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Brevibucca frugicola n. sp., a saprophagous nematode found in a rotting peach fruit.

By T. Goodey, D.Sc.

(*Institute of Agricultural Parasitology, St. Albans.*)

Early in 1938 the writer received a tube of nematodes from Mr. Colin Smee, Entomologist, Department of Agriculture, Zomba, Nyasaland, collected from a rotting peach fruit in which he had been trying to hatch fruit-flies. According to Mr. Smee's letter the worms had formed masses in the fruit and had also appeared on the surface as waving threads or tendrils composed of numerous eelworms massed together. The material had been fixed in glycerine alcohol and consisted of numerous adult males and females as well as large numbers of larval forms. Fixation was not good as although the gross morphology and anatomy was fairly easily discernible, some of the finer structural details were not as clear as one could have wished.

By the slow evaporation of the liquid containing the worms the latter were finally left in glycerine. Numbers of adult males and females were then mounted and studied further. In general structure they were found to resemble members of the genus Rhabditis; the oesophagus having the shape and structure characteristic of that genus with a valve apparatus in the posterior bulb. In the females the vulva was found to be practically equatorial in position with the gonads paired and reflexed. In the males the anterior end of the testis was reflexed, the tail was seen to have lateral alae with numerous papillae whilst the spicules were paired and similar to each other. The most noticeable difference from Rhabditis, however, was the short and rather broad buccal cavity in which feature they closely resembled nematodes placed by the writer in the genus *Brevibucca* Goodey, 1935. To this genus, therefore, they are assigned as a new species.

The generic diagnosis of *Brevibucca* given in the 1935 paper is too restricted in scope to embrace the present species. It was based on the type species (up to now the sole species of the genus) which showed certain rather specialized anatomical features such as the posterior position of the vulva, the presence of a pair of gland cells connected to either side of the uterus and the fact that the males possessed dissimilar spicules. The following amended generic diagnosis of the genus is therefore put forward.

Brevibucca Goodey, 1935 : Amended generic diagnosis. Rhabditidae, mouth surrounded by six lips each bearing at least one small papilla. Buccal cavity a little longer than wide, sides straight or slightly convex in optical section. Oesophagus Rhabditis-like; the corpus composed of a cylindrical pre-corpus leading to the corpus, i.e. the medial oesophageal bulb; isthmus followed by final bulb containing a valve apparatus. *Female,* vulva median or posterior, gonads paired or single. *Male,* testis single, anteriorly outstretched or reflexed, spicules paired, similar or dissimilar, gubernaculum present. Caudal alae present or absent; caudal papillae numerous.

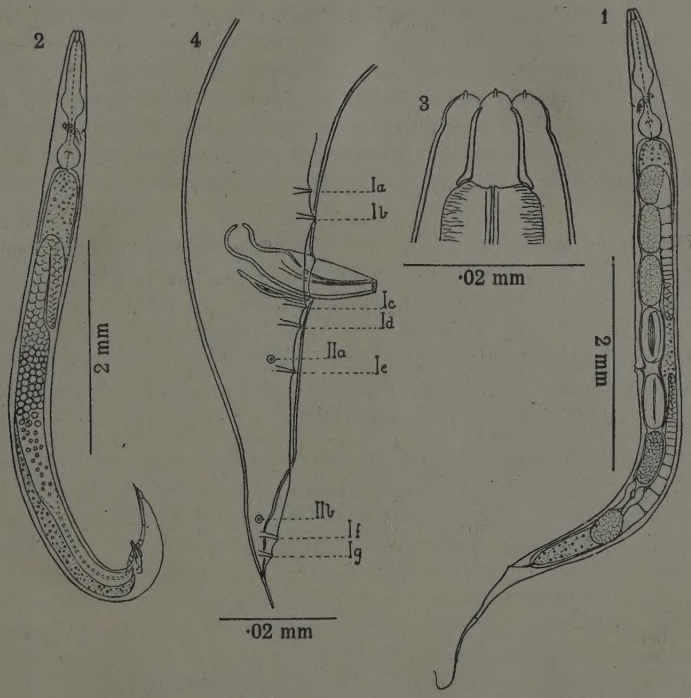
Brevibucca frugicola n. sp.

Dimensions : *Female,* length, 0·57 mm. to 0·78 mm., $a=17·6-19.1$, $\beta=4·5-6$, $\gamma=4·6-5·2$, V=41% to 56·6%. *Male,* length, 0·42 mm. to 0·7 mm., $a=14-20$, $\beta=3-5$, $\gamma=8·5-10·6$, spicules, $28\mu-34\mu$ long, gubernaculum, $13\mu-15\mu$ long. In both sexes the body tapers slightly in the oesophageal region towards the head end. The female tail is long and filiform. The male tail is shorter than that of the female but tapers to a fine point. Cuticle with very fine transverse striae borne on numerous longitudinal striations. Lateral fields about one-third the width of the body. Mouth surrounded by 6 roundly conical lips, each provided with a very small forwardly-directed papilla. Buccal cavity a little longer than broad and consisting essentially of a protostom; a distinct cheiloston with cheilorhabdions not being discernible. The protorhabdions composing the walls of the protostom (fig. 3) taper a little anteriorly and are turned slightly inwards. They are about 12μ to 14μ long and are expanded basally where they rest upon the anterior end of the oesophagus. Owing to the poor fixation of the material, it has been impossible to determine whether a telostom with distinct telorhabdions is present. The anterior part of the oesophagus, i.e. the pre-corpus, is almost cylindrical in shape and is muscular in structure. It merges with the rather distinct corpus or median oesophageal bulb which is also muscular. The isthmus is crossed by the nerve ring and then enlarges into the posterior oesophageal bulb which contains a valve apparatus. The excretory pore is situated at about the level of the nerve ring. The intestine extends throughout the body and is connected with the anus by a distinct rectum.

Female.—The vulva is medially situated; in some specimens being a little pre- and in others a little post-equatorially placed. The gonads are paired, opposed and reflexed. Eggs are apparently laid in an

advanced stage of development since females have been found containing fully embryonated eggs. There is a minute lateral caudal papilla or phasmid on either side of the tail situated at about the halfway point.

Male.—The single testis extends well forwards in the body and its anterior end is reflexed for a short distance. The tail (fig. 4) tapers



Brevibucca frugicola n. sp.

Figs. 1 & 2.—Adult female and male in lateral view to show general shape and structure.

Fig. 3.—Head end highly magnified to show shape of buccal cavity.

Fig. 4.—Male tail in lateral view showing disposition of caudal ala and papillae on one side. Only one of the pair of spicules is shown.

gradually to a sharp point. On each side of the tail there is a distinct but rather narrow membranous ala arising a little in front of the heads of the spicules and extending practically to the tip of the tail but not surrounding it. There appear to be 9 pairs of caudal papillae arranged in two groups, one sub-ventrally situated consisting of 7 pairs of papillae and

the other laterally situated consisting of 2 pairs of papillae. In the former group all the papillae extend to the edge of the alae. Of these papillae, 1a and 1b are pre-anal, 1c and 1d are just post-anal, 1e is a little further back. Then there is a considerable gap before we come to 1f and 1g, which occur quite close to the tip of the tail. The two lateral papillae are both post-anally placed; 11a occurring close to 1e whilst 11b lies a little in advance of the level of 1f and 1g. The spicules are paired, arcuate and similar in shape. Anteriorly, each is cephalated by constriction. From the shoulder region the shaft tapers gradually to the tip which is fairly stout, blunt and flat. On the anterior side of each spicule a wing-like expansion extends from the shoulder to the tip. The gubernaculum is simple, pointed at each end and rather thicker in the middle.

The new species differs from *Brevibucca saprophaga* in a number of anatomical features, the chief of which are that in the female the vulva is median and the ovaries paired, opposed and reflexed whilst the tail is long and filiform. In the male there are caudal alae with numerous caudal papillae arranged differently from those of *B. saprophaga,* and the fact that the spicules are similar to each other in shape and size and are not dissimilar as in *B. saprophaga.*

Biology.—It is probable that the worms are saprophagous in habit since they were found living in the rotting tissues of a peach fruit. In support of this it may be mentioned that when examined under high magnification many of the adult worms were seen to have large numbers of bacteria in the intestine. It is of interest to note also that numbers of them (probably larvae) collected on the surface of the material and there formed tendril-like masses which gently waved about. It seems reasonably certain, by analogy with the behaviour of other nematodes which manifest a similar propensity to form such masses of waving larvae, that it is by this means that they achieve dispersal to other media favourable to further growth and development. Insects such as flies or beetles on visiting a medium with such larvae on the surface come into contact with them with the result that the larvae in some way become attached to various regions of the insects and so are readily carried to other sources of food. In this connection the observations of Aubertot (1923) may be cited. He found that a culture medium consisting of a purée of boiled potato moistened with a little vinegar attracted vinegar flies, *Drosophila confusa,* when placed in the open well above soil level. There was thus no possibility of it

becoming contaminated from the soil. In the course of a few days, after being visited by vinegar flies, the medium contained an abundance of nematodes which were later identified as *Rhabditis pellio* Schn. and which must have been brought to it by the flies. By the 21st day the surface of the culture showed numerous tufts or bundles of 3rd stage larvae which exhibited a waving or nodding movement. Flies walking on the surface of the culture and coming into contact with these larvae were found to have masses of them attached to the underside of the body; on the prosternum, in the intercoxal spaces, on the first abdominal sternite and in the occipito-thoracic groove. When these larvae-bearing flies visited a fresh, moist medium the larvae absorbed moisture and fell on to the medium where they rapidly underwent further development to the adult stage and completed the life cycle. Similar transport of the encysted larvae of other nematodes by insects is well known in the case of *Rhabditis coarctata* Leuck. whose larvae attach themselves to various species of *Aphodius* and other dung inhabiting beetles [see Triffitt & Oldham (1927)]. More recently Bovien (1937) has added considerably to our knowledge of this subject and has described a number of species of *Diplogaster* and *Rhabditis* the larvae of which are spread by means of insects. It seems probable, then, that *Brevibucca frugicola* is also a species whose larvae are carried on the bodies of flies since it occurred in peach fruits infested with fruit flies and its larvae exhibited the phenomenon of forming waving tufts or bundles on the surface of the medium.

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On *Pungentus thornei* n. sp., a new dorylaimid nematode from soil.

By T. Goodey, D.Sc.

(*Institute of Agricultural Parasitology, St. Albans.*)

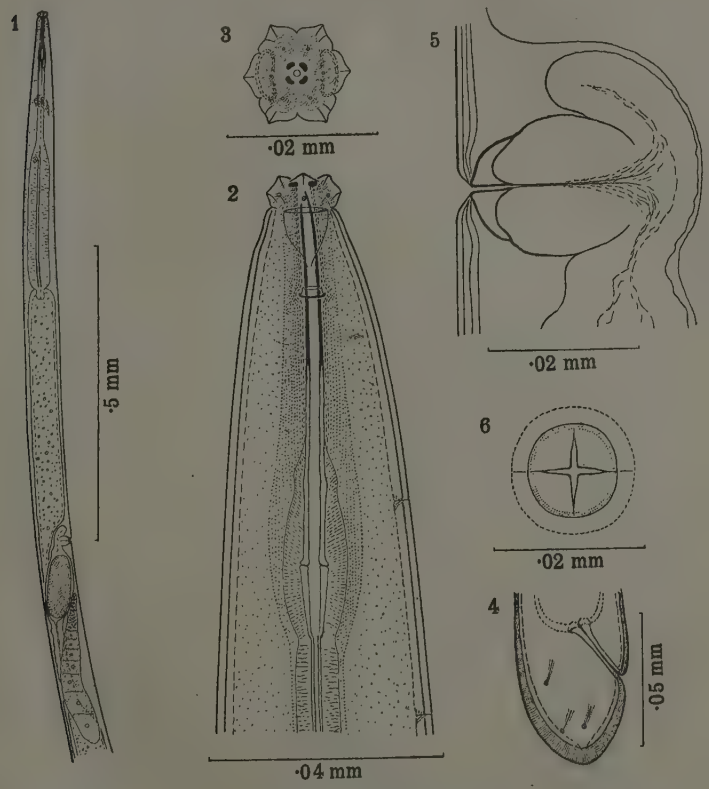
In water extracts of turf from one of the meadows at this Institute made May and November, 1941, several specimens of a new dorylaimid nematode were obtained. They were noticeable even under low magnification by reason of the elongated mouth spear and the shape of the tail. Females only have been found. Study of freshly-killed and well preserved specimens showed that they belong to the genus *Pungentus* of Thorne & Swanger (1936). As, however, they do not agree structurally with any of the species described by Thorne & Swanger (l.c.) and by Thorne (1939), a new species is erected for their reception. The worms are named in honour of Dr. Gerald Thorne, who in the two monographs just cited has added so materially to our knowledge of the soil nematodes belonging to the superfamily Dorylaimoidea.

Morphology.

Dimensions: *Female*, length, 1·7 mm. to 2·22 mm., $a = 31-39$, $\beta = 3{\cdot}7-5$, $\gamma = 68-7$, $V = 39\%-45\%$. Average dimensions based on 13 specimens, length, 1·91 mm., $a = 33{\cdot}8$, $\beta = 4{\cdot}3$, $\gamma = 69$, $V = 41.8\%$.

Under low magnification the cuticle appears to be smooth but when examined under high powers it is seen to carry very fine transverse striae. These are rather more evident on the tail than elsewhere. The body tapers gently anteriorly; posteriorly there is practically no decrease in width and the short tail is bluntly conical or rounded in outline. The head is offset by constriction and is composed of 6 rather prominent lips. An *en face* view of the head (fig. 3) shows that, in all, there are 16 labial papillae consisting of a peri-oral circlet of 6 which are forwardly directed and 10 others which are antero-laterally directed and are situated as follows, 1 on each lateral lip and 2 on each of the other four lips. Immediately within the oral aperture and surrounding it is a series of 4 cutinized plates. The amphids are goblet-shaped with a wide opening at the level of the head constriction. The mouth leads into a vestibule in the form of a long hexagonal tube. Within this lies the spear the tip of which, when retracted, lies just within the mouth aperture. The spear varies from 30μ to 36μ in length. It is not quite straight but is slightly concave on its dorsal side. As in other

dorylaimid forms, the orifice at the spear tip is directed dorsally. There is a guiding ring surrounding the spear situated about halfway down the vestibule. It is an extensible structure, shaped very much like a crush opera hat the crown of which extends forwards as the spear is



Pungentus thornei n. sp.

Fig. 1.—Fore part of body under low magnification, showing general shape of oesophagus and position and extent of gonad.

Fig. 2.—Head and spear, highly magnified, in lateral aspect showing spear, guiding ring and spear extension.

Fig. 3.—*En face* view of head ; the outline of the amphids is shown by dotted lines under each lateral lip.

Fig. 4.—Tail in lateral aspect.

Figs. 5 & 6.—Lateral and *en face* views of vulva. In fig. 5 the vagina and its connections with the uterus are shown.

extruded. Spear and guiding ring are sloughed along with the cuticle during ecdysis and the new spear, already formed in a cell lying well forward in the oesophageal region, migrates forward and takes its place. Attached to the spear base is the spear extension which has a length of about 45μ. It appears to consist of two parts; an anterior, making up about three-quarters of its length, and a posterior part. The anterior part has practically parallel sides but with a slight swelling at about two-thirds of its length and the walls end in small expansions. The latter fit on to the similarly expanded ends of the posterior portion of the spear extension the walls of which taper gradually towards the median axis and are continued into those forming the lining of the oesophagus. Surrounding the posterior three-quarters or so of the spear extension is a well developed fusiform sheath of muscle. The oesophagus is typically dorylaimoid in shape and consists of a narrow neck region followed by the elongate posterior enlargement. The cardiac cells at the junction of the oesophagus and intestine form a hemispherical mass protruding into the beginning of the intestine. A pre-rectum is present but is rather indistinct in outline. It appears to be comparatively short and varies in length from two to three times the body width at the anus. The tail is roundly conical in shape. The cuticle clothing it is thicker than on the rest of the body and the fine striations are more easily visible here than elsewhere. There appear to be 3 caudal papillae on each side of the tail as shown in fig. 4. In the mid-ventral line of the body and extending from a short distance in front of the anus right up to the level of the nerve ring is a row of very fine papillae. They are somewhat irregularly and widely spaced. On one adult specimen 28 of them were counted. They are not present on the dorsal side of the body. Each appears to consist of a small conical mound of hypodermis in the centre of which is a very fine core with its point occupying the centre of a minute shallow pit in the cuticle. The gonad is single, posterior and reflexed with the tip of the ovary lying just behind the level of the vulva. The latter is shaped like a Maltese cross; the orifice being both transverse and longitudinal with 4 lips surrounding it. The lips are thick and hyaline and, *en face*, appear like 4 triangles whose apices surround the vulval orifice. The vagina leads into the body at right angles to the longitudinal axis. It is short, has rather thick walls and opens into the uterus which extends a little in advance of the level of the vulva as a short blind sac. Posteriorly the uterus reaches for a variable distance, varying from 4% to 8% of the body length, and then joins the ovary as the latter is flexed forwards

in the body. The uterine walls are cellular in structure and there may be from 1 to 3 eggs in the uterus at a time.

The new species, in having a single reflexed gonad, resembles *Pungentus monohystera* Thorne & Swanger, 1936, but differs from it in its greater length, in having a well set-off head with 6 distinct papillate lips as compared with an indistinct head and obscure lips and papillae of *P. monohystera*. The female tail of the latter has only 2 caudal papillae on each side as compared with three in *P. thornei*. In the shape of the head the new species resembles *P. angulosus* Thorne, 1939, but is at once differentiated from this species by its single gonad; the sexual organs in *P. angulosus* being paired and opposed. There seems to be no doubt that the worms belong to a species previously undescribed and they are accordingly named *Pungentus thornei* n. sp.

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Journal of Helminthology, Vol. XX, *Nos.* 1/2, *August*, 1942, *pp.* 9–24.

Observations on *Mononchus tridentatus, M. brachyuris* and other species of the genus *Mononchus*.

By T. GOODEY, D.Sc.

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STUDIES carried out by the writer during the past few years on various species of the genus *Anguillulina*, associated chiefly with the roots of grasses, have resulted in the collection of numerous other species of soil nematodes including many representatives of the predatory genus *Mononchus*. Amongst these are specimens of *Mononchus tridentatus* de Man, 1876, *M. brachyuris* Bütschli, 1873, two species in which males as well as females are known, *M. muscorum* (Duj. 1945), *M. punctatus* Cobb, 1917, *M. papillatus* Bast., 1865 and *M. minor* Cobb, 1893; all species in which females only are known. Investigation of these forms has resulted not only in the discovery of facts concerning their feeding habits but also of certain interesting anatomical features which have hitherto remained incompletely elucidated in members of this genus.

Mononchus tridentatus de Man, 1876.

This species has been found many times in aqueous extracts of turf taken from pastures at this Institute. It is a fairly abundant species occurring both in spring and autumn and males are practically as numerous as females. It occurs here in greater numbers than any other species of *Mononchus* so far encountered. Adults and pre-adult larvae have voracious appetites and feed indiscriminately on various other species of soil nematodes, of which large numbers are consumed, as well as on small oligochaets.

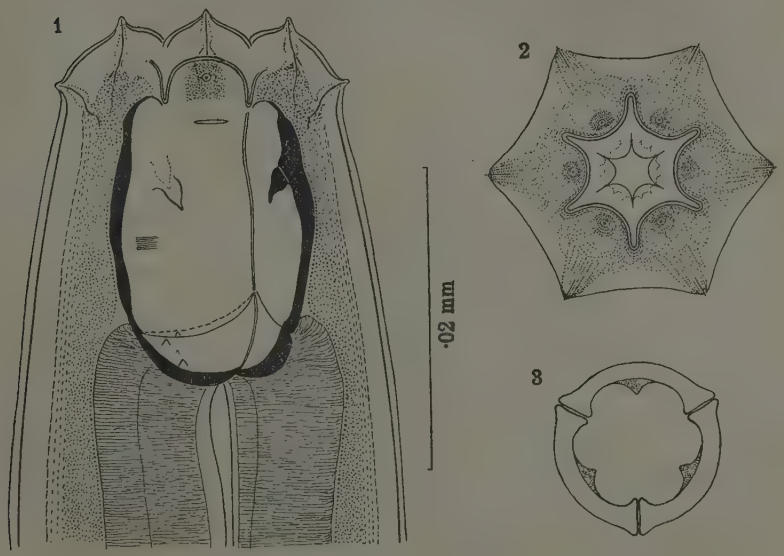
Although originally described by de Man in 1876 and again by him in 1884, little further information on the anatomy of the worms was published until de Man (1921) gave some further details on anatomical features. There are a number of records on the occurrence of the species in Germany and Switzerland which are listed by Micoletzky (1921). Although Cobb (1917) speaks of it as " widespread in Europe in moist soil " and as " rather common," he gives a drawing of the head end taken from de Man (1876), and it seems probable that he had no first-hand acquaintance with the species as he mentions two anatomical features on which exact information is lacking, namely whether caudal glands and amphids are present which he could scarcely have failed to observe had he examined the worms himself. de Man (1921) on Pl. IV, fig 8, shows that he had found amphids but he does not mention caudal glands. Detailed observations on a good number of fresh and well preserved specimens of both sexes which the writer has made enable him to present new information on the presence of caudal glands, the structure of the female gonads, the occurrence of ejaculatory glands in the male and on certain other anatomical details as well as facts concerning the feeding habits of this species.

Morphology.

Dimensions :—*Female,* length, 2·11 mm. to 3·19 mm. $a=27-35$, $\beta=4-5$, $\gamma=8·5-12·7$, $V=58·8\%-68\%$. *Male,* length, 2·2 mm. to 2·57 mm., $a=27·8-34$, $\beta=4·1-4·6$, $\gamma=10·3-13·4$. These measurements are somewhat smaller than those given by de Man whose figures are 3·6 mm. and 2·7 mm. as the lengths of female and male respectively. The principal proportions set out above agree very well, however, with those given by de Man.

The body tapers slightly towards the anterior end but the lip region, which is offset by constriction, is comparatively wide and the anterior

end is rather flat. The head is made up of 6 lips which when viewed *en face* give it a hexagonal outline (fig. 2). Each lip bears two innervated papillae; one directed anteriorly and the other laterally. The mouth aperture is comparatively small and is surrounded by 6 transparent leaf-like lips. It is lined by rugose thickenings which project inwards and have somewhat the appearance of leaf-crown elements as double or triple knobs on the inside of each lip. The mouth leads into the buccal cavity which, when viewed in lateral aspect (fig 1) is seen to



Mononchus tridentatus.

Fig. 1.—Lateral view of head, highly magnified.

Fig. 2.—*En face* view of head.

Fig. 3.—Transverse section of buccal cavity at level of base of teeth, latter shown stippled.

be barrel-shaped with stout hyaline walls on which are found 3 prominent, sharp, backwardly directed teeth situated a little anterior to the equator of the cavity. By focusing deeply into an *en face* view of the head it can be seen that the wall of the cavity is composed of 3 equal sectors (fig. 3), one dorsal and two subventral in position. These abut on one another by the flattened faces of their expanded ends and each bears a tooth centrally placed on its inner face. A lateral view

of the head shows a fine ring-like constriction at the base of each tooth. The inner wall of the buccal cavity is not perfectly smooth but bears fine cross striations; a feature which Cobb noted in several species of this genus. de Man (1921) drew attention to the fact that in sexually immature forms the three teeth are situated towards the lower end of the buccal cavity. The writer can confirm this as amongst the specimens studied by him are a number of pre-adult larvae in which the three teeth occur close to the lower end of the main barrel-like region of the buccal cavity. A cup-shaped amphid is present on each side of the head laterally situated a little in advance of the level of the buccal teeth. The three sectors comprising the barrel-like walls of the buccal cavity terminate where the anterior end of the oesophagus joins on to it. Here their lower ends articulate with three plates which form the floor of the cavity; one plate being dorsal and two subventral in position. The plates can be seen to be distinct from the walls of the buccal cavity in most specimens but their separate identity is more easily recognisable in mounted specimens which have become rather compressed and in which the several parts composing the buccal cavity have become separated from one another. The inner edges of the plates frame a triradiate space in the base of the cavity and this is continued into the lumen of the oesophagus. Towards the outer margin of each of the two subventral plates, but not of the dorsal one, are what appear to be two small triangular prominences. These are the so-called basal denticles figured by de Man but whereas he figured and described two such prominences there are really four. They are not solid in structure and are not true teeth but are angular foramina in their respective plates. It is possible that they are the outlets of subventral oesophageal glands as was suggested by Cobb (1917, p. 142) for similar so-called " teeth " or denticles in other species.

The oesophagus is slightly swollen at its junction with the base of the buccal cavity. It is very muscular in structure and the lining of the lumen is very prominent. It is crossed by the nerve-ring at about one-quarter to one-third of its length from the anterior end. There appear to be numerous oesophageal glands forming a sort of net-work of finely granular tissue amongst the muscles. Some of these glands, possibly dorsal ones, open into the lumen of the oesophagus through a fine pore in the lining nearly midway of its length. The cells at the junction of the oesophagus and the intestine form a kind of flattened plate. The intestine is tessellated in appearance and is composed of a pavement of polygonal cells each containing a nucleus surrounded by fine granules

or globules in the protoplasm. The lumen of the intestine is roomy and is thus well fitted to accommodate the numerous captured nematodes on which this species mainly feeds. No particular species of free-living eelworm seems to be selected as food, but whatever proves to be available or convenient for capture is devoured. The following species have been recognised by the writer within the intestine of mounted specimens, *Panagrolaimus rigidus, Cephalobus* spp., *Pratylenchus macrophallus, Anguillulina agricola, Plectus parietinus, Rhabditis* spp., and the larvae of various species of Dorylaims. The latter forms are sometimes so bulky that a single specimen may occupy almost two-thirds or three-quarters of the length of the intestine. Buccal spears of Dorylaims as well as spicules and oesophageal valve apparatuses are easily recognisable within the intestinal contents in many examples. In addition to nematode material one occasionally finds a specimen which has been preying upon small oligocheat worms recognisable by the characteristic setae. Three females in the writer's mounted specimens show large numbers of leptomonad flagellates within the intestine in addition to numerous nematodes. They were not seen in the worms whilst still alive but are clearly recognisable as flagellates in the specimens mounted in glycerine. They have the same fusiform appearance and size as the leptomonad flagellates figured and described by Goodey & Triffitt (1927) in the intestine of *Diplogaster longicauda*, being from 10 to 12μ long.

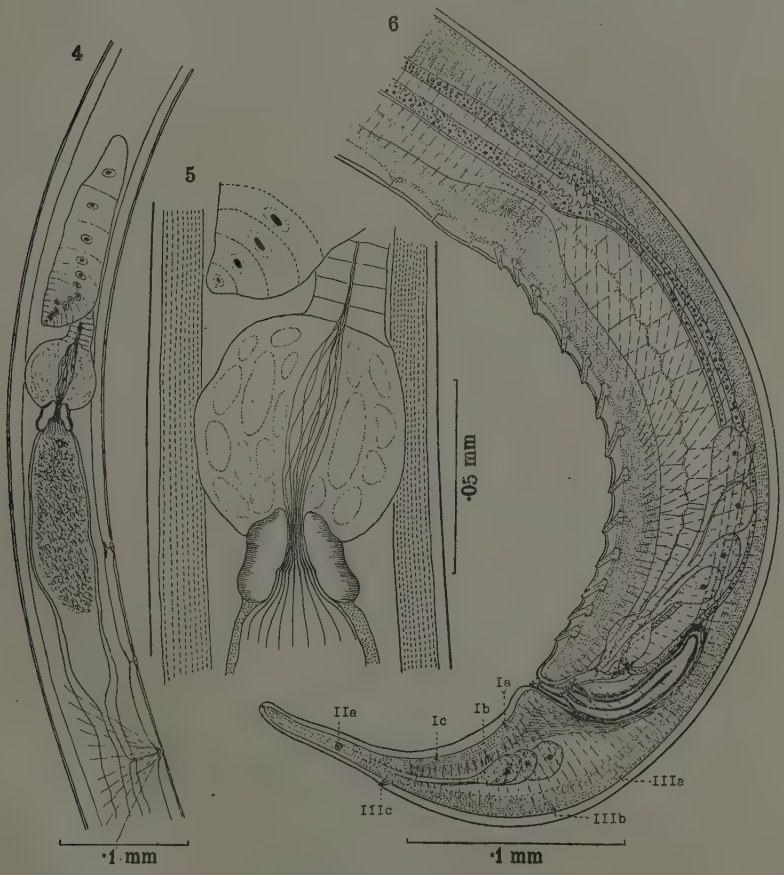
Female.—The ventrally arcuate tail tapers evenly to the tip which carries the outlet of the caudal glands in a terminal position. There are three of these glands lying one behind the other in the central tissues at the base of the tail just behind the anus, and each has a nucleus surrounded by vacuolate protoplasm. The narrow ducts from the glands extend to the end of the tail where they empty into an ampulla before opening to the exterior. There are three caudal papillae on each side of the tail, two of which are laterally placed and one dorso-laterally. Of the former, one is situated at about one-quarter to one-third the length of the tail behind the anus and the other at about the same distance from the end of the tail. The dorso-lateral papilla is placed at about two-thirds the length of the tail from the tip.

In the vicinity of the vulva, in the mid-ventral line, the writer has found a few irregularly distributed very small pit-like papillae with a fine central core. The vulva is rather small and slightly raised; the lips bearing cuticular thickenings. The vagina leads inwards at right

angles to the ventral surface and opens into the common tube from which the anterior and posterior uteri are given off. Radiating from the vulva are a series of dilator muscles which are attached to the dorso-lateral body wall. The gonads are paired, opposed and reflexed. Each uterus contains, as a rule, two or three eggs, but occasionally as many as four may be found in each. The presence of eggs in the uteri usually has the effect of obscuring the structure of each gonad owing to the compression and displacement of its various regions. Occasionally, however, a specimen may be found in which eggs are absent from the uteri and the detailed structure of the parts composing each gonad can then be clearly seen. de Man (1921) found such a female in which between the uterus and the commencement of the oviduct there is a spherical dilatation (shown in his Pl. IV, fig. 8c). It is clear from his drawing that the spherical dilatation represents the swollen end of the oviduct into which a portion of the end of the uterus protrudes for a short distance. This end part of the uterus is really the sphincter which is herein described and figured in greater detail in figs. 4 and 5. Proceeding from the point where the vagina opens into the common uterine tube, it can be seen that each uterus consists of a cellular tube which is about three times as long as the body width in this region. It expands slightly in its distal half and here frequently contains a mass of sperms. Terminating the uterus and separating it from its oviduct is a well developed sphincter, shaped like a truncated cone, having muscular walls and a hyaline cuticularized lining. The latter consists of fine strands or fibres which arise from the inner wall of the uterus, rather like the framework of a parasol, which then converge into the centre of the sphincter, where they are slightly thickened, and become more refractive in appearance. They then pass on and form the lining of the swollen end of the oviduct into the substance of which the narrower end of the sphincter is often more or less pressed. The swollen end of the oviduct is cellular in structure but the outlines of the individual cells composing it are indistinguishable from one another and the whole region presents a granular vacuolate appearance. It is capable of great expansion to accommodate eggs on their passage to the uterus. The oviduct narrows down and continues as a cellular tube to its junction with the reflexed ovary where it expands to accommodate each ovum as it becomes fully formed. Each ovary consists of a strand of ova gradually diminishing in size to the rather blunt terminus lying alongside its oviduct.

So far as the writer is aware, the foregoing structures consisting of

a sphincter terminating each uterus and separating it from each oviduct have not previously been described in *Mononchus* species. The writer has, however, found somewhat similar well developed anatomical features in the gonads of *M. brachyuris*. Cassidy (1931) in her drawing



Mononchus tridentatus.

Fig. 4.—A female gonad, under moderate magnification showing arrangement of various regions. Alimentary tract purposely omitted.

Fig. 5.—Junction of uterine sphincter and swollen end of oviduct, highly magnified.

Fig. 6.—Male tail showing disposition of the various organs, supplements, papillae and ejaculatory glands.

of the female of *M. brachylaimus* figures a sphincter at the anterior end of the forward uterus though she does not describe it. In specimens of *M. muscorum, M. punctatus, M. papillatus* and *M. minor* which the writer has studied, though both gonads show a swollen end to the oviduct and the uteri narrow down before joining with it, a well defined sphincter does not appear to be present.

Male.—The tail tapers rather steeply just behind the cloacal aperture to the level of the third subdorsal pair of caudal papillae from which point to the tip it is almost cylindrical. This region of the tail is very variable in length; being much longer in some specimens than in others. The tip of the tail carries the outlet of the ducts from the three caudal glands. As in other species of *Mononchus* males of which have been described, the male of *M. tridentatus* has paired testes which are opposed and outstretched. The anterior testis extends less than halfway down the intestine to the end of the oesophagus. The vas deferens lies ventral to the intestine and in its terminal region often has stout cellular walls as shown in fig. 6. It tapers posteriorly to a narrow ejaculatory duct and this opens into the anterior wall of the rectum which thus becomes a cloaca. A constant and fairly prominent feature of this region of the body is a row of 4 (rarely 5) ovoid or rounded gland cells which are found on each side of the body and lie over part of the vas deferens or the intestine, or both, just below the body wall. Each has a single nucleus surrounded by granular protoplasm. Posteriorly each cell narrows down into a delicate walled duct. The four ducts in some specimens appear to unite in pairs to form two ducts and these lead backwards into a kind of vesicular enlargement or dilatation of the lateral wall of the cloaca. Whether the ducts join up in pairs is very difficult to determine accurately as in many specimens they overlie one another and it may be that they remain distinct throughout their course. The exact limits of each lateral dilatation of the cloacal wall and the precise manner in which the ducts open into it are also difficult to define but it seems clear that the glands open into the cloaca anterolaterally and not into the vas deferens or the ejaculatory duct.

To judge from de Man's figure of the male tail in the original description of this species (1876), i.e. his fig. 50b, it would seem that the foremost pair of these glands was seen by him as they are clearly indicated in outline, though they are not described in the text. Four gland-like bodies, probably homologous with those just described are also figured by Bütschli (1876) in his drawing of the male tail of what he took to be *M. truncatus* Bast., but to which Cobb (1917) applied the

name *M. obtusus*. Bütschli, however, did not describe these structures as glands. Micoletzky (1921) p. 351, figures the outlines of 4 roundish gland cells lying lateral to the posterior region of the vas deferens in *M. studeri* Steiner, and says that they probably open into the ductus ejaculatorius or into the cloaca. He does not, however, figure or describe the ducts. At the same time it is clear that he recognised their relationships as he says they may correspond to the cloacal glands of other genera. Similar glands, four on each side of the body, are also present in the males of *M. brachylaimus* Cobb as is evident from the drawing and description of this species given by Cassidy (1931). She says :—"At the anterior end of the spicula a filamentous prolongation is seen passing anteriorly and embracing four large sized rounded nucleated cells which lie anterior and dorsal to it." She does not figure or describe the ducts. It is shown later on that in the males of *M. brachyuris* Bütschli two large gland cells, similarly situated, are present on each side of the body with ducts opening antero-laterally into the cloaca. It would seem highly probable that such glands (perhaps best considered as ejaculatory glands) are regularly present in males of the genus *Mononchus*. A note is appended later on the homologies of these glands.

The posterior part of the body, for some distance in front of the cloacal opening, is very muscular. The chief muscle bundles encircle the body but where the ventral row of supplements begins the muscle bands become backwardly directed and in this region the substance of the body wall on the ventral side becomes raised into a mound which compresses the vas deferens. At any rate this is so in fixed specimens in which there is a marked ventral flexure of this part of the body. In the mid-ventral line there is a row of 13-14 stout supplements whose tips protrude a little on the outside of the cuticle and whose broader bases are inserted in the substance of the body wall. Each is innervated centrally. In advance of these there are a further 10 to 13 mid-ventral papillae each of which has a fine core arising from a flattish prominence of the body wall. The spacing of these papillae is quite irregular, and they extend only about halfway along the body. In some specimens the hindmost 4 or 5 of them are placed nearer together.

The tail bears 7 pairs of postanal papillae; 3 pairs being subventral, Ia, Ib, and Ic; 1 pair is lateral, IIa, and 3 pairs are subdorsally placed IIIa, IIIb, and IIIc. They are situated as shown in fig. 6, and all are fairly easily discernible with the exception of IIIa and IIIb which can only be found by careful focusing on the surface of the body

under the oil-immersion. The ventrally arcuate spicules are paired and each is enclosed in a muscular sheath attached to the anterior end of which is a pair of retractor muscles the forward ends of which are inserted in the dorsal body wall. Posteriorly a stout bundle of muscles connects the spicular sheath to the body wall behind the cloacal opening and no doubt serves in the protrusion of each spicule. The tip of each spicule appears to be trifid and down the middle of the organ runs a strengthening strand. The gubernaculum is a complicated structure consisting of an underlying keel and the more conspicuous parts which are found in a lateral position a short distance from the cloacal aperture. Of these, the central portion is a bifurcate piece on either side of which are cuticular processes, the lower of which has a latero-dorsal prolongation connecting with the keel. The bifurcate central piece of these lateral elements seems to be a characteristic feature of the gubernaculum in males of certain other species of *Mononchus*.

Mononchus brachyuris Bütschli, 1873.

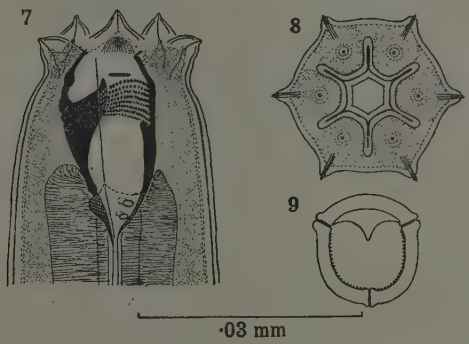
In the literature dealing with the genus *Mononchus* there are several species which possess a funnel or goblet-shaped buccal cavity provided with a large dorsal tooth and with rows of very small denticles or rasp-like prominences on areas of the lateral walls. Cobb (1917) described a number of such species, many of which were made synonyms of *M. brachyuris* by Micoletzky (1922). In the case of several of these forms females only are known and the specific differences between them are based on very slight differences of size and detailed structure of the head and tail. The male of *M. brachyuris* was not described by Bütschli but was first described and figured by de Man (1876). The writer has, with some hesitation, identified certain worms as belonging to this species because of their general agreement with published accounts of it and also because the males studied agree very well with the drawing of the male tail figured by de Man (1876), fig. 51. The examples investigated were obtained from turf both of a heavy loam and of a light sandy character.

Morphology.

Dimensions: *Female*, length, 1·64 mm. to 2·12 mm., $a=30-41$, $\beta=3·2-4·1$, $\gamma=72-90$, $V=60\%-65·6\%$. *Male*, length, 1·64 mm. to 2·06 mm., $a=33-46$, $\beta=3·5-3·8$, $\gamma=53-68$.

The body tapers but slightly at the head end and the lip region is offset by a constriction. Both in lateral aspect and in an *en face* view,

the head can be seen to be made up of 6 prominent, roundly conical lips each of which carries two papillae; one anteriorly and one laterally directed. The mouth aperture is hexagonal in outline when seen *en face*. It leads into the buccal cavity which is made up of three sectors, one dorsally and two subventrally placed. As shown in figs. 7 and 9 the dorsal sector carries a very large tooth with a forwardly directed point, whilst on the walls of the subventral sectors, at the level of the tooth shaft, there are 7 or 8 rows of small rasp-like denticles. On the ventral side there are also two rather blunt and not very prominent teeth, one of either side of the ventral line. The base of the cavity narrows down considerably and is occupied by three plates which appear to be fused to the three sectors making up the body of the cavity, one dorsal and two subventral. They are shown in fig. 7 by cross hatching.



Mononchus brachyuris.

Fig. 7.—Lateral view of head, highly magnified.

Fig. 8.—*En face* view of head.

Fig. 9.—Transverse section of buccal cavity, showing tooth on dorsal sector and small denticles on inside of 2 subventral sectors.

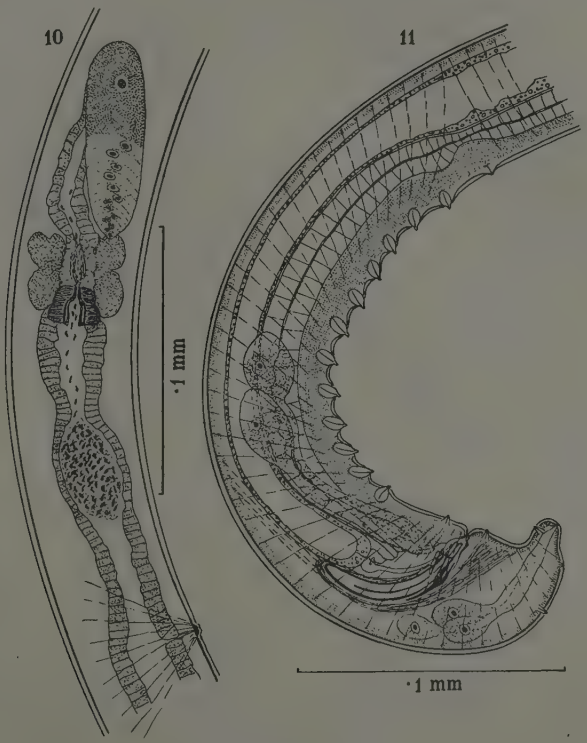
As in *M. tridentatus*, each subventral piece towards its outer margin carries 2 foramina, but instead of being triangular, as in *M. tridentatus*, they are ovoid or elliptical in outline. The oesophagus and intestine are typical and call for no special description. None of the writer's mounted specimens reveal nematodes or nematode remains in the intestinal lumen but according to Cobb and other observers this species preys on other small nematodes. In passing, it may be mentioned that two specimens, 1 male and 1 female, amongst the writer's material show an extensive invasion of the intestinal cells by some kind of sporozoan parasite, some

examples of which, probably in an advanced stage of development, are free in the lumen of the gut.

Female.—The female tail is short and is shaped like that of the male (see fig 11). It carries four pairs of papillae situated similarly to those of the male tail; 1 pair subventral and immediately postanal, 1 pair just anterior to the tip of the tail and 2 subdorsal pairs. There are 3 caudal glands the outlet from which is slightly dorsal to the extreme tip of the tail. The gonads are paired, opposed and reflexed. The general arrangement of the regions comprising one of them is shown in fig. 10, where it can be seen to follow the same plan as that of *M. tridentatus*. The vulva opens into a thick walled vagina which leads at right-angles into a common uterine tube. Each uterus has fairly stout cellular walls and, in spermatized individuals, contains a mass of rather large spermatozoa towards its distal end. It terminates in a sphincter the lining of which is clear and hyaline in appearance, and though apparently made up of 6 or 8 ridges, it is not fibrillar as in *M. tridentatus*. The narrower portion of the sphincter is usually partly enclosed by the swollen end of the oviduct through the centre of which the lining of the lumen is continued. As in *M. tridentatus*, this portion of the oviduct has dense spongy walls and is capable of great extension to accommodate an egg on its passage to the uterus. In the unexpanded state it always carries an equatorial constriction. The sphincter appears to play some part in controlling the passage of spermatozoa into the narrower region of the oviduct. They can frequently be seen not only in the main mass within each uterus but also in small numbers within the narrow part of the oviduct. It is probable that sperms also pass into the narrow oviduct in females of *M. tridentatus* but, in spite of careful examination, the writer has not been able to satisfy himself of their presence there. The sperms of *M. tridentatus* are, however, smaller than those of *M. brachyuris* and small numbers of them are more difficult to locate. The fertilization of the egg must take place within the narrow part of the oviduct in both species as eggs complete with shells have been found within the swollen end of the oviduct in both species.

Male.—In fixed and mounted specimens the tail region of the male is more or less strongly flexed ventrally as shown in fig. 11. The latter shows the chief anatomical features and by comparison with fig. 6 the similarity of the general ground plan of structure common to the two species as well as the differences between them can readily be seen. There is a midventral row of 12 hyaline, ovoid, innervated supplements

the tips of which protrude a little through the cuticle. These supplements do not commence immediately in front of the cloacal aperture but at a short distance anterior to it, whilst immediately preanal there is a smaller papilla. Anterior to the supplements and also in the



Mononchus brachyuris.

Fig. 10.—A female gonad, moderately magnified, showing arrangement of parts and uterine sphincter at junction of uterus and swollen end of oviduct.

Fig. 11.—Male tail in lateral view, showing principal organs, etc., and 2 large ejaculatory glands.

midventral line there are one or two finer papillae rather irregularly distributed. The tail carries 4 pairs of papillae situated as shown in fig. 11, 2 pairs being subventral and 2 pairs subdorsal in position. The paired spicules, each in a muscular sheath, have practically the

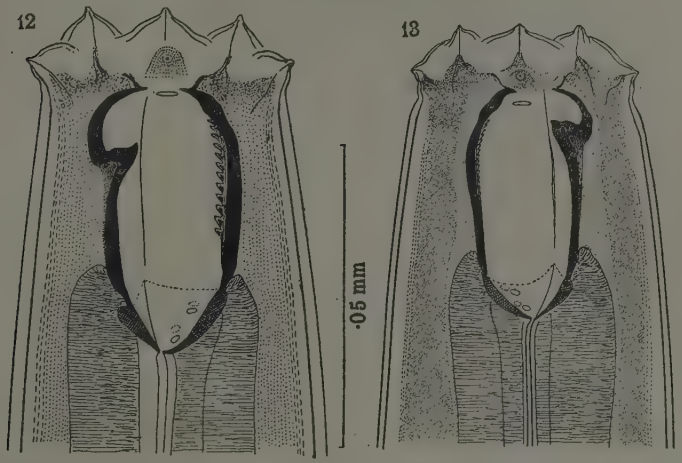
same shape as those of *M. tridentatus* but are smaller and the complicated gubernaculum is rather like a smaller edition of that in *M. tridentatus*. The final narrow part of the vas deferens opens into the anterior floor of the rectum quite close to the end of the intestine. Instead of 4 ejaculatory glands on each side of the body, there are only two, situated as shown in fig. 11. Each gland cell is a rather large ovoid or clavate body containing a fairly distinct nucleus surrounded by finely granular protoplasm. Posteriorly each cell narrows down to a delicate walled duct and the two ducts run side by side, partly overlying the ejaculatory duct, to open into a lateral dilatation of the cloacal wall. As in males of *M. tridentatus*, the exact course of the ducts, particularly in their terminal regions, is very difficult to determine accurately, but it seems clear to the writer that they empty antero-laterally into the cloaca and not into the ejaculatory duct.

Additional Notes on Anatomical Features.

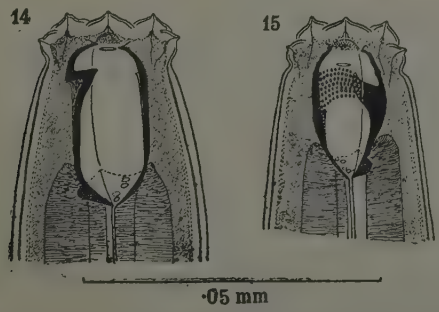
1. *Basal plates of buccal cavity and foramina of subventral ones.*— Careful study of the buccal cavity in *M. tridentatus* led the writer to the view that the posterior part of it is not just a continuation of the three sectors comprising the main walls but consists of three separate plates whose inner edges frame the triradiate lumen of this region. In this species the articulation or loose connection of these basal plates with the lower ends of the three sectors composing the barrel of the cavity is fairly easily made out. The presence of two triangular foramina in each of the subventral plates, the so-called basal denticles of de Man, has also been noted earlier. These findings led to the close examination of such other species of *Mononchus* as were available, including *M. muscorum* (Duj.), *M. punctatus* Cobb, *M. papillatus* Bast., *M. brachyuris* Bütschli and *M. minor* Cobb (see figs. 12 to 15).

In these drawings the thickened parts of the basal plates are hatched in to show their difference from the main sectors of the buccal cavity. Many earlier accounts of members of this genus are illustrated by drawings which show these parts as having a different appearance from the main part of the buccal cavity but, as far as the writer is aware, their distinctness has not previously been emphasized. It is not suggested that in all species the parts concerned are actually separated from the main part of the cavity since in such species as *M. brachyuris* and *M. minor* the basal parts seem to be fused with the main sectors, but in all the species so far examined by the writer

fainter or clearer lines of demarcation of these regions can be discerned under high magnification. With regard to the two foramina in each



Figs. 12 & 13.—Head end of *M. muscorum* and *M. punctatus* respectively, showing structure of buccal cavity, etc. Basal plates (cross-hatched) and foramina in subventral plates.



Figs. 14 & 15.—Head end of *M. papillatus* and *M. minor* respectively, showing structure of buccal cavity, etc. Basal plates (cross-hatched) and foramina in subventral plates.

of the subventral plates, these have been found to be present in all the species so far examined.

2. *Homology of male ejaculatory glands.*—The presence of four

nucleated gland cells on either side of the body in the region of the posterior part of the vas deferens in males of *M. tridentatus, M. obtusus, M. studeri* and *M. brachylaimus* and of two gland cells in *M. brachyuris* whose ducts open laterally into the cloaca calls for some comparison with similar glands in other nematodes. Chitwood (1930) pointed out that multicellular glands, so-called "cement glands," had been described first by Schneider in 1866 in males of *Rhabditis strongyloides* and much later by Cobb in 1925 in males of *Rhabditis icosiensis*. He showed that in *R. strongyloides* these large glands, which lie on either side of the terminal region of the vas deferens, have practically the same cellular structure as the vas deferens. They may, in fact, be considered as outgrowths from the latter, and the lumen of each opens into that of the vas deferens some distance anterior to the rectum. Chitwood found, in addition to these large multicellular glands, a pair of smaller gland cells situated on either side of the body posterior to the larger ones. Each consists of two nucleated cells and the ducts from these open into the ejaculatory duct just anterior to or at the level of the rectal glands. It seems reasonable to suggest that the gland cells described above as occurring in the males of *Mononchus* species are probably homologous with the smaller ejaculatory glands in *Rhabditis strongyloides*, though, as has been pointed out, their ducts open into the cloaca and not into the ejaculatory duct itself.

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On two new Coenuri from Africa, and a note on the development of the hooks.

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Multiceps macracantha n. sp.

The material on which this species is based consists of a single coenurus which was recovered from the body cavity of a tame white rat in Southern Rhodesia in 1930. When it was made available to me, through the kindness of Dr. R. T. Leiper, C.M.G., F.R.S., two scolices had been mounted as press preparations and the rest of the material was bottled in glycerine and alcohol.

There was a small well-developed adventitious cyst, roughly spherical, measuring 13 mm. by 10 mm. by 10 mm. This contained the parasitic cyst which measured 5 mm. in diameter. It had apparently carried, in addition to the two mounted specimens, only 4 more scolices. These had all evaginated themselves during fixation and were very obvious as prominent bodies standing out from the surface of the cyst. These buds all measured at least 3 mm. long and even then showed signs of considerable contraction. There was no evidence of strobilar segmentation. In 3 of them, the rostellum was invaginated but the fourth was a favourable specimen with the rostellum well expanded and the crown of hooks well in view. This specimen was measured. The total length of the bud was 3·7 mm.: the scolex measured 825μ long by 477μ broad. The suckers were about 240μ in diameter. The rostellum measured 210μ in diameter and carried the typical taeniid hooks arranged in two circles, the large hooks alternating with the small ones. The hooks were examined from the press preparations. There were 38 on each slide. They are of 2 sizes and extremely large, being comparable in size to those of *T. laticollis* and *T. taeniaeformis*, a fact which distinguishes them from all other species of Multiceps. The shape, too, is characteristic.

The large hooks are strong, massive structures from 377μ to 391μ long, the means being 379·2μ and its standard deviation 5·431, with a well-developed blade of only slight curvature: it is broad and stout and the tip is slightly blunt. It measures from 328μ to 348μ long on its dorsal side with a mean of 337·4μ and its standard deviation is 7·348.

Ventrally it measures from 138μ to 147μ, the mean and standard deviation being 143·3μ and 3·375. The guard is strong and rounded, lying at an angle with the blade: it has the general appearance of an equilateral triangle with the corners rounded off. The handle is turned dorsally and is very short: it is usually notched on both its dorsal and ventral edges. The tip, in contrast with other species of *Taenia* and *Multiceps*, is not rounded but is indented giving the appearance of two blunt knobs, usually approximately equal but sometimes the indentation is not median but slightly dorsal when the knobs become dissimilar. The union of the blade with the handle is very well marked ventrally, less so dorsally but it can be followed along the whole lateral surface of the hook for the blade is slimmer than the base. Like the large hooks the small ones are strong, sturdy structures, measuring from 249μ to 256μ. The blade is quite strongly curved, from 111μ to 117μ long ventrally, slightly blunt at the tip. The guard is blunt and rounded, not deeply bifid and lies in a line with the base of the blade. The handle is relatively larger than in the large hooks and is blunt: both dorsal and ventral edges are almost straight. There is, however, a slight depression on the lateral flat surface, almost like a thumb print. The union of the blade with the base is difficult to distinguish dorsally, but is an obvious landmark ventrally and it can be traced across more than half the width of the surface.

At a single glance it is quite obvious that these hooks have not been described before. The only taeniid species which carry hooks of a comparable size are *T. laticollis* and *T. taeniaeformis*. The last named species has a cysticercus for a larva whereas the new species has a coenurus. Further, the shape of the handle is different as are also the relative proportions of the hook. Turning then to *T. laticollis*, we find this to be an incompletely described species. The larval stage is not known and therefore the possibility that it is a coenurus cannot be excluded. The length of the large hooks varies from 380μ to 420μ but though a description has been given by Rudolphi (1819), Diesing (1850), Leuckart (1856), Lühe (1910) and abstracted by Hall in 1919, yet none appears to remark on a very obvious indentation at the tip of the handle. It seems unlikely therefore that Rudolphi's *T. laticollis* can be the adult form of this coenurus from Rhodesia. It is therefore suggested that this material represents a species hitherto undescribed and the specific name *macracantha* is attached to it, to indicate the very striking size of the hooks.

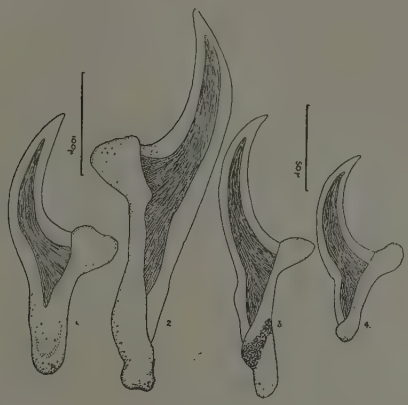
The information about this species may be summarized as follows :—

Species—*Multiceps macracantha* n. sp.

Definitive Host—Not known.

Larval Host—White Rat, in the body cavity.

Distribution—S. Rhodesia.



Figs. 1 & 2. Hooks of *M. macracantha* n. sp.
Figs. 3 & 4. Hooks of *M. otomys* n. sp.

MULTICEPS OTOMYS n. sp.

The second species of *Coenurus* that has to be described was a subcutaneous parasite of a mouse *Otomys erroratus* in Pretoria and was located near the eye. Only a portion of the parasitic cyst was available but from this portion it could be seen that the scolices were arranged in rows. The material may have been somewhat shrunk by the fixation but the scolex measured 850μ long by 410μ wide. The rostellum had a diameter of 270μ and the suckers of 180μ. There was no strobilar segmentation. There were the usual two circles of hooks on the rostellum. The large hooks were remarkable for their fragility : a very dainty touch was necessary to avoid fracture. They ruptured very easily at a line just below the union of the handle with the blade. That this was not due to minor damage during handling is shown by the

fact that this region is obvious even in hooks that have not been touched by either a needle or a cover slip, for here the cuticle is deeply pitted and fissured. It is just possible that this appearance may not be typical for the hooks arise from two areas of chitinization which finally meet at this point, a subject that will be discussed later in this article. It is possible that chitinization was not complete, but against this is the fact that in all other species of *Taenia* and *Multiceps*, the hooks seem to develop and reach maturity at an early age.

The large hooks in this species measure from 162μ to 182μ long, with a mean of 171·4μ and a standard deviation of 2·497. They have a well curved blade measuring from 71μ to 81μ ventrally, the mean and standard deviation being 74·32μ and 2·317, and from 128μ to 143μ dorsally, the mean and standard deviation being 131·64 and 1·975. There is a deep depression on the dorsal edge. The guard is set at an angle with the blade and tapers slightly to form a blunt tip. The handle is broad and relatively long, turned dorsally at the tip. The ventral edge is usually fairly straight or evenly convex; the dorsal edge tends to be more sinuous and invariably carries a deep hollow before the end. The small ones are more distinct than is usually the case among the Taeniid species. They measure from 108μ to 120μ long and have a strongly curved blade. There is a well marked angular depression dorsally about 2/3 along the length of the blade. The guard is comparatively large and massive. The handle is short, usually rounded and blunt but in a few cases was slightly drawn out and tapering. The most striking feature about the small hooks, however, is the fact that the union of the blade with the base is very distinct along the whole lateral surface of the hook.

These hooks are distinguished from all other species of *Multiceps* hooks by their size and relative proportions. They are larger than those of any species of *Taenia*, whose life history is still obscure. Among the species of *Multiceps* the large ones approximate in size to those of *M. gaigeri* but on the whole are larger and the handle is relatively shorter. The small hooks are smaller than those of *M. gaigeri* but they would approximate to the lower limits. However, there is a very small handle. The larva of *M. gaigeri* is typically a parasite of Ungulates, which is rather a far cry from the rodents. It does not seem unreasonable therefore to suggest that this is a distinct species and the specific name of *otomys* from its larval host is assigned to it.

Summarizing this information :—

Species—*Multiceps otomys* n. sp.

Definitive Host—Not known.

Larval Host—Mouse, *Otomys erroratus,* in the subcutaneous tissues.

Distribution—Pretoria.

The material of these two species has been deposited in the helminthological collection of the London School of Hygiene and Tropical Medicine.

The Development of the Taeniid Hook.

These hooks appear at an early stage of development in the larva—(see Clapham & Peters, 1941, whose investigations treated with strict regard to statistical principles demonstrate that the larval hook in certain species of *Multiceps* show no evidence of further growth in the definitive host). That this is also the case among the more common species of *Taenia* and *Multiceps* is borne out by similar results, as yet unpublished, on various species of which material was available.

However, during the examination of a very large amount of material, particularly of actively proliferating coenurus material, there has come to light both juvenile forms and monstrosities, from which it has been possible to piece together something of the method of development. So far it has not been possible to examine the earliest stages of development before chitinization has begun for they seem to be passed through quickly and the material that has been sectioned has been either too young or too old. But the later stages make an interesting observation.

The two types of hook do not arise in precisely similar circumstances. In immature scolices it can be seen that the large hooks develop from at least two centres of chitinization. The blade and guard portion appears early and the handle is added afterwards from a second centre and gradually grows forward to meet the blade. Thus there is a stage, which can be seen quite frequently, in which the large and the small hooks appear to form a single circlet, each hook being of approximately the same size and shape. Alternate hooks, however, have a wedge of chitin in close proximity. As this grows forward the blade also grows to complete its normal size until the fully developed hook is apparent. All the intermediate stages from a very small wedge of handle chitin to full union have been seen.

Further evidence that this is the normal method of development is afforded by certain monstrous forms. A fairly high percentage of such forms have been observed in coenuri that have developed in unusual hosts, e.g. *Coenurus cerebralis* in man and *C. serialis* in a variety of odd hosts. On many occasions the large hook has developed without its attendant handle ; in others no blades or guards of either sized hooks have developed though the handles of the large ones are present. This suggests that some cause has operated to abolish either the blades and guards of all the hooks or the handles of the large ones. This further suggests that the blade and guard of the large hook and the entire small hook arise from a common ring of chitinization and the handles of the large hooks form a distinct one.

There was seen a young scolex in which the armature appeared as wedges of chitin of a roughly triangular shape, regularly arranged round the rostellum. It may be that this was a stage in the early development of the armature, but as no intermediate stages have been seen, it cannot be certain.

These observations bring to mind the case of the species *Taenia monostephanos*. This was first reported by von Linstow in 1905, who first regarded it as a specimen of *T. laticollis* and later as a distinct species on the ground that it has only a single crown of hooks and these of an atypical shape. They have a much reduced handle, the result being like that of a rose thorn. If this is a normal state then the material must represent a distinct species and one not entirely typical of the genus *Taenia*. It is, however, possible that von Linstow was dealing with an immature form in which the handle was represented by small masses of chitin still not fused with the main portion of the hook. The fact that he described 19 hooks—an odd number—is not a real difficulty for specimens frequently lose one or more hooks during handling.

Summary.

Two new species of *Multiceps* are described. *M. macracantha* n. sp. a parasite of the white rat in Rhodesia, is distinguished by the possession of very large hooks. *M. otomys* n. sp. from a mouse, *Otomys erroratus* in Pretoria, has fairly large hooks of a characteristic appearance and shape.

The fact that the large taeniid hook appears to develop from at least two centres of chitinization is noted.

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On identifying *Multiceps spp.* by measurement of the large hook.

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THE genus *Multiceps* has come under intense observation recently and the validity of the various species has been considered. The hook, being of a chitinous nature and therefore presumably of a less variable disposition than most other points, has been used for specific diagnosis, though as has been shown earlier, even this structure is not entirely satisfactory. But there is no other structure that is more satisfactory. When the information has been available, the blade length has been used as this is the most constant part of the hook. The small hook is of little or no diagnostic value. In many cases, however, only the range of the total length has been known.

The species *serialis* was the first to be examined critically and from material available it was found to have a wide distribution both biologically and geographically for coenuri from a gelada baboon, *Theropithecus gelada*, from a porcupine *Hystrix longicauda*, from *Myopotamus sp.*, from rats in Accra and in Nigeria, from a gerbille *Gerbillus hirtipes* and from mice in London have all proved basically indistinguishable from the rabbit form. The large hooks range in length from 110-175μ with a mean of 136·06μ and a standard deviation of 3·448. The small ones measured from 68-120μ long. The blades of the large ones had a ventral length of from 43-64μ with a mean of 57·3916μ and a standard deviation of 2·34. The dorsal length of the blade varied from 70-114μ, the mean being 99·618μ and the standard deviation 3·13.

M. multiceps next came under consideration and it was found that using hook measurements as a basis for specific diagnosis there is no reason for separating the two species. Using a large quantity of material, comprising three separate and unrelated coenuri and taking at random 225 scolices from these, the total length of the large hook was found to vary from 120-170μ with a mean of 142·2μ and a standard deviation of 4·199. The blade lengths varied from 53-73μ ventrally with a mean of 62·501μ and a standard deviation of 3·073. Dorsally they varied from 94-120μ long with a mean of 105·08μ and a standard deviation of 2·739. The small hooks varied in length from 76-130μ. When either the blade lengths or the total lengths of the large hooks were examined by the laws of statistics, the results from *M. multiceps* and from *M. serialis* fall into the same series. The shape of the hook is indistinguishable in the two species, though it is generally stated in the text-books that the guard of the small one in *M. serialis* is bilobed and in *M. multiceps* it is grooved or notched. But having drawn close on 1,000 hooks with a camera lucida, it can be stated with some assurance that this difference is only one of degree. There are small hooks in *M. multiceps* that are very deeply notched and among those of *M. serialis* some that are only slightly bifid and there comes a state when it is not possible to say whether a guard is deeply grooved or lightly bifid. Given only hooks, with no other information, it would not be possible to make a sure diagnosis. There are other alleged differences—the shape of the mature segments and the thickness of the strobilae, both of which however will vary with the degree of expansion and the method of fixation. The number of calcareous granules varies not only among individuals of the same species but also in different parts of the same individual. Even the arrangement of the scolices within the coenurus is by no means fixed in serial rows in *M. serialis* and in groups in *M. multiceps*. Then too it is said that *M. multiceps* in the larval stage never induces the development of an adventitious cyst by the host while *M. serialis* always does. But this is a host reaction and the fibrocytic potentialities of the cerebral tissues may be slighter than those of the intermuscular connective tissues. None of these differences then seem to be sufficiently fixed or important to justify elevating to specific rank. The final and only true test would of course be the transmission of *Coenurus cerebralis* through a dog to a rabbit and of *C. serialis* through a dog to a sheep, which transmission is however impossible during war time. Positive results from it would necessarily be proof of the singleness of the two species but a negative result would

Measurements of large hooks in *Multiceps* spp. (measurements in μ).

Species.	Number of Hooks Examined	Total Length	Mean	Stand. dev.	Blade lengths					
					Dorsal side	Mean	Stand. dev.	Ventral side	Mean	Stand. dev.
M. multiceps (cerebral form)	225	120–170	142·2	4·199	94–120	105·08	2·739	53–73	62·501	3·073
M. serialis	600	110–175	136·06	3·448	70–114	99·618	3·13	43–64	57·392	2·34
M. glomeratus		96–105 90–110								
M. packii		140–150								
M. spalacis		162–170								
M. clavifer		122–132 114–120								
M. polytuberculosus ...		70								
M. lemuris		125								
M. ramosus		110–132								
M. multiceps (comprehensive figures)	825	110–175	137·738	4·349	70–120	101·241	3·03	43–73	58·907	3·794
M. twitchelli		189–198								
M. radians (T. brauni)		85–105 130–140 95–100								
M. gaigeri	75	145–180	159·906	6·403	105–118	111·746	2·144	61–69	64·33	1·197
M. macracantha	20	377–391	379·2	5·431	328–348	337·4	7·348	138–147	143·3	3·375
M. otomys	20	162–182	171·4	2·497	128–143	131·64	1·975	71–81	74·32	2·317

not necessarily prove that there are two valid species, for we have in helminthology the classic example of *Ascaris lumbricoides* in man and pig, now accepted by all zoologists. Here is species, morphologically indistinguishable in the two hosts but biologically quite distinct. There is a similar condition to be found in *Necator americanus,* two strains of which have become adapted to life in either man or the pig but not in both. It is quite possible that we have a like condition among coenuri i.e. that there is a large widely spread species, *M. multiceps* composed of two or more races. The Ungulate race finds its way to the central nervous system where it takes on its individual form without causing the development of an adventitious cyst by the host. The race that passes its larval stage in a rodent however, finds the most convenient habitat to be intermuscular connective tissues and here it usually forms a neat, more or less spherical cyst, surrounded by a strong fibrous tissue capsule formed by the host. There may be even more than two races. We know that " *C. serialis* " can behave atypically in other hosts and even on occasions in the rabbit for it is capable of considerable proliferation and budding and may result in the formation of daughter cysts and/or fine ramifications. It would seem therefore to be a very fluid species. As the name *M. multiceps* has priority over *M. serialis* by 67 years, the latter name becomes a synonym of the older one. Leske applied the name *M. multiceps* to the cerebral form of the species in 1780.

There are also other species of this genus which it may be convenient to consider now. First there is *M. glomeratus* described by Railliet and Henry in 1915 from a gerbille, *Gerbillus hirtipes* in Tunis, and by Turner and Leiper from man in West Africa in 1919. Re-examination of the hooks of the human material and comparison of them with the drawings of the original material make it almost certain that neither specimen was mature. In an earlier article it has been described how the hook develops from at least two centres of chitinization and both these examples show hooks in an incomplete stage of development before the handle has become attached to the blade. That being the case there is no reason to suppose that this material can justifiably be called a distinct species. Further as " *M. serialis* " has been recovered from both man and the gerbille, this is a further reason why these two samples should be incorporated into the larger species and the name *glomeratus* go into abeyance.

Baylis described a cyst which had been removed from a human subject from the Belgian Congo. It had previously been considered

by Taramelli and Dubois. Baylis was unwilling to assign it to any particular species for it was intermediate between *M. multiceps* and *M. serialis*. The large hooks resemble those of *M. multiceps* and measure 140-155μ long, while the small ones resemble those of *M. serialis* and measure from 110-115μ. But if our first supposition is correct, then the difficulty disappears and the material can be assigned to *M. multiceps*. He points out too that *M. packii* first described by Christenson in 1930 from *Lepus americanus phaenotus* in Minnesota cannot be distinguished from *M. serialis*. The large hooks have a total length of from 140-150μ and the small ones of from 96-100μ. These figures bear out his theory and so it seems natural that this name should disappear also. The parasite lay in the pericardial sheath of the host, an unusual site, but in all its significant parts was identical with the known species.

M. spalacis was described as a larva from *Tachyoryctes splendens* in Abyssinia. The length of the large hooks being from 162-170μ falls into the range of *M. multiceps* as do also the small ones for they measure from 125-130μ long. It seems to resemble in many ways the material from the Belgian Congo which was classified without much difficulty as *M. multiceps* and there seems no reason for treating this material any differently and it can therefore be added to our comprehensive species. Diesing had earlier described a form with a single circlet of hooks from *Georhychus capensis* which was considered by Joyeux as an aberrant form of this species and which is therefore unsuitable material to use for the description of a new species.

M. clavifer was described by Pagenstecher in 1877 and again by Railliet & Mouquet in 1919 from *Myopotamus sp.*, Pagenstecher's material had large hooks which measured from 122-130μ long (amended measurements by Baltzer) and the small ones from 84-93μ. The material examined by Railliet and Mouquet gave hook measurements of from 114-120μ for the large ones and from 90-98μ for the small ones, but their hooks were either immature or abnormal for the handle and guard scarcely existed and they cannot be considered as normal material. They call their material *M. clavifer* because of the nature of the host but *M. multiceps* has been found in *Myopotamus sp.* and these figures and descriptions suggest *M. clavifer* is a synonym of *M. multiceps*.

M. polytuberculosus was described rather superficially by Mégnin in 1880 from a tame jerboa, *Jaculus sagitta*, but his drawings and descriptions are not complete and it is difficult to form a judgment on it. The hooks apparently only measured 70μ and 50μ long which is much smaller

than anything else that we have but his drawings are not easy to decipher. It was mainly remarkable for its budding, a condition which we now know to be comparatively common.

Coming finally to coenuri which have been described from primates, we have several records of "*M. serialis.*" Schwartz described a coenurus from a baboon which he had no hesitation in assigning to the species *M. serialis* on morphological grounds but as he found it was impossible to produce the next generation experimentally in a rodent, he considers it to be a distinct strain physiologically. The present observer has evidence that "*M. serialis*" may occur in baboons. Man may also be a host of this species.

M. lemuris has been described by Cobbold from a ring tailed lemur of Madagascar which died in the Zoological Gardens many years ago, where it had formed a multilocular cyst involving the liver and lungs. Railliet & Marrulaz describe a coenurus from *Macacus sinicus* (*Macaca radiata*) which they consider to be an example of this species and which they call *C. ramosus* however. In each case the size of the large hooks being from 110-132μ (R. & M.) and 125 (Cobbold) approximates closely to the lower limits assigned to *M. multiceps* while the small hooks being of from 89-92μ long (R. & M.) and 80μ (Cobbold) are of a fair average size and this material can therefore be assigned to the species described by Leske.

The general impression that one gets after close examination of hooks of coenuri is that *M. multiceps* is of wide biological and geographical distribution and that it may contain several different strains which seem to establish themselves in different hosts, where they adopt an individual form and behave in their own characteristic way. How far these strains are distinct physiologically, it is of course impossible to say without experimental trials, except in the case described by Schwartz, who found his material did not develop in rodents. There may or may not be strains among the rest of the material.

There remain 5 other species of *Multiceps* which ought to be considered. *M. twitchelli* described by Schwartz from a porcupine *Erethizon epixanthum myops* in Alaska would seem to be a valid species for it possesses large hooks, the measurements of which do not overlap those of any other species. The large hooks measure from 189-198μ long and the small ones from 155-163μ long. It has an interesting and curious dendritic habit of budding and branching which has not been described in any other species.,

M. radians from a mouse in France is small in every way and the hook has a short handle which seems, however, to be fully formed and therefore this may be a valid species. The large hook measures from 85-105μ long and the small one from 58-75μ long, and it has been suggested that it closely resembles *T. brauni,* alleged to have a coenurus stage in a gerbille in Egypt. The hook measurements of *T. brauni,* however, are difficult to understand, being given at one time as from 130-140μ for the large ones and from 85-90μ for the small ones. At another time the figures are given as from 95-100μ and 70-75μ respectively. This suggests that several different samples are confused under one name. The material may even become classified as *M. multiceps* for the larger measurements coincide very well with those of the material examined here.

M. gaigeri has a large hook which measures from 145–180μ long and the small one from 103-160μ long. These measurements were taken from material available here and give a much smaller minimum than is usual. This low minimum was due to a single scolex, taken however from the same coenurus as the others, and the scolex showed no sign of malformation or immaturity. Altogether 225 hooks were measured of each size. The mean of the total length of the large hooks was 159·906μ and its standard deviation was 6·403. The blade lengths gave the following results :—dorsally the blade measured from 105-118μ; mean 111·746μ and its standard deviation was 2·144 : ventrally the measurements varied from 61-69μ with a mean of 64·33μ and a standard deviation of 1·127. As, however, previous authors have given no indication of the number of hooks measured, nor of the frequency of the various lengths within the limits, it is impossible to compare any two sets of figures. These new figures overlap those of *M. multiceps* quite considerably at their lower limits, though the shape of the handle is somewhat distinct, being rather longer than is found in the type species. Yet it is worth noting that *M. gaigeri* occurs mainly in India and Ceylon where rabbits are said to be unknown and the rodent fauna scanty. The coenurus may settle either in the central nervous system or in the intermuscular connective tissues. The adult is supposed to resemble closely that of *M. multiceps* and the larva is certainly not quickly distinguished from that of "*M. serialis.*"

The only other two species left for consideration were described by the present observer in a previous article in this journal, *M. macracantha* from a rat is outstanding by the very great size of its hooks and by the bifid shape of the handle of the large one. The total

length varied from 377-391μ, the mean being 379·2μ and its standard deviation 5·431. The dorsal side of the blade measured from 328-348μ with a mean of 337·4μ and a standard deviation of 7·348. Ventrally the blade measured from 138-147μ with a mean of 143.3μ and a standard deviation of 3·375. The hooks are more than twice the size of anything else in this genus.

M. otomys can be distinguished by the size, shape and curious markings on the large hooks. The blade is relatively longer than in other species. The total length varies from 162-182μ with a mean of 171·4μ and a standard deviation of 2·497. Dorsally the blade length measured from 128-143μ long with a mean of 131·64μ and a standard deviation of 1·975. Ventrally it measures from 71-81μ long and its mean and standard deviation were 74·32μ and 2·317 respectively.

In these records there have been published, in regard to any particular measurement, the range of the particular length, together with its mean and standard deviation. Maximum and minimum lengths alone have practically no diagnostic value. If, however, the other two values are added, then later samples of material can be correlated with previously described material and true values and relationships assessed. Lengths of the small hooks are added here but their means and standard deviations are omitted as it has been shown earlier that the small hook is so variable in all its component parts as to have no use in the diagnosis of species.

Summarizing these observations we may say that the number of species of *Multiceps* can be reduced on morphological grounds and *M. multiceps* becomes a comprehensive species with a wide host and geographical distribution. Many of the older and less well known species can reasonably be accommodated within its confines and *M. serialis*, though it is an old and well established species, can also go there. The species is very fluid and is probably evolving in several directions according to the nature of its intermediate host. Thus it may appear in several situations, as a normal cyst or as a much branched structure, and it may or may not induce the development of an adventitious cyst by the host. Because of its great variability it is safe to say that specific diagnosis of a sample of coenurus material is not an easy matter and there should be great hesitation before creating a new species. A new species should only be made from material that shows a very striking difference and some strongly distinguishing marks, and as much material as possible should be examined, because even a single

coenurus shows great variation. All measurements should be examined by the methods of statistics.

AMENDED DESCRIPTION OF THE HOOK OF *M. MULTICEPS*.

Total length varied from 110-175μ with a mean of 137·738μ and a standard deviation of 4·3486 in the large hooks. The blade lengths vary from 43-73μ long ventrally, the mean being 58·907μ and its standard deviation 3·794. Dorsally the blade measures from 70-120μ long with a mean of 101·241μ and a standard deviation of 3·03.

Site of the coenurus in the intermediate host—central nervous system, intermuscular connective tissues, abdominal cavity, thoracic cavity and pericardial sheath.

Intermediate hosts.—Primates, including man, ungulates, rodents.

Geographical distribution.—Europe and the Mediterranean Islands, Iceland, Siberia, India, Japan, Australia, New Zealand, Egypt and the Southern Mediterranean Coast, Union of S. Africa, S.W. Africa, U.S.A. and South American Republics.

The genus probably contains not more than 6 species :—*Multiceps multiceps, M. radians, M. twitchelli, M. gaigeri, M. macracantha* and *M. otomys,* all of which bear some distinct feature by which they can be clearly distinguished, though the case of *M. gaigeri* may bear further consideration.

The larval stage of *M. multiceps* can develop in man and in his domestic stock as well as among wild mammals, a point which must be considered for preventive measures. It has been recognised in man nearly a dozen times in the intermuscular connective tissues and twice in the brain, but it may easily have been overlooked in the brain, on other occasions. There are records of human infestation, usually under the name of *M. serialis,* from the Belgian Congo, France (several records), Gold Coast, West Africa and England.

I am much indebted to Dr. R. T. Leiper, C.M.G., F.R.S., who put all this material at my disposal and to Dr. B. G. Peters who has given me very much assistance with the statistical side of the work. Both of them have given me much helpful advice.

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On the lethal Effect of Sulphur Dioxide on eelworm Cysts adherent to Seed Potatoes.

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Whilst there are many factors which contribute to the spread of the potato eelworm, *Heterodera rostochiensis*, vel *H. schachtii* auctorum, to uninfected land, the advisability of ensuring that seed planted on eelworm-free soil is free from adherent cysts is obvious. Whilst much might be accomplished by formulating regulations to prevent the planting on clean land of seed grown in infected areas, it would be distinctly advantageous, as well as more economical in seed, if some method were available to remove or destroy their adherent infection. The effectiveness of washing to remove the cysts is dealt with in a separate paper.

The use of sulphur dioxide to destroy the cyst is based on some unpublished preliminary experiments by Professor Leiper which had given promising results. The subsequent investigations made by me at his request may be conveniently grouped under three main heads :

(*a*) Action of sulphur dioxide on the free cysts.

(*b*) Action of sulphur dioxide on cysts attached to seed potatoes.

(*c*) Action of sulphur dioxide on the seed potatoes.

The "in vitro" Action of Sulphur Dioxide on *H. rostochiensis* cysts.

To investigate the action of sulphur dioxide on the cysts, the gas was mixed with air and used in the presence and in the absence of water. The chemical was obtained from a siphon of the aqueous chemical and was mixed with air in a gas mixing apparatus. When its action in the absence of moisture was being investigated the gas mixture was bubbled through concentrated sulphuric acid whereas when its action under moist conditions was being determined, the damp gas from the gas mixing apparatus was passed directly into the treatment chamber in which wet cysts were supported on damp filter paper. In all cases the cysts were supported in the middle of the chamber, well away from the walls and the gas passed through for the requisite time. After treatment the cysts were removed and soaked in water for 14 days at

24°C. Six batches of fifty cysts were then selected at random from each treatment group and subjected to the action of root excretion for seven days. Larvae which hatched out were removed daily and counted, the root excretion being renewed each day. In all twelve treatments and a control involving experiments in the presence and in the absence of water, three concentrations of sulphur dioxide as well as two periods of time, were performed. The results are set out in Table 1.

TABLE I.

The Effect "in vitro" on Cysts of *Heterodera rostochiensis* of Exposure to Sulphur Dioxide—air mixtures.

Treatment.	Time.	Total hatch over seven days.						Total.
Control ...		173	242	191	130	129	128	993
1 : 8 moist...	10 mins.	0	1	0	0	1	0	2
	20 mins.	0	0	0	0	1	0	1
1 : 16 moist	10 mins.	0	2	0	1	0	0	3
	20 mins.	1	0	1	0	0	0	2
1 : 30 moist	10 mins.	1	2	2	2	1	1	9
...	20 mins.	1	1	0	3	0	1	6
1 : 8 dry ...	10 mins.	13	16	10	13	22	14	88
	20 mins.	16	8	1	9	13	14	61
1 : 16 dry ...	10 mins.	19	22	11	51	23	22	148
	20 mins.	15	19	12	15	15	14	90
1 : 30 dry ...	10 mins.	13	34	30	23	21	20	141
	20 mins.	20	11	29	35	26	34	155

It will readily be seen that the number of larvae hatching out of cysts treated with sulphur dioxide was much less than the number hatching out of the untreated controls. If we compare the total hatch for the six batches of untreated cysts with the maximum total hatch for any treatment group of six batches we are really comparing 993 with 155—a difference of 838. Accepting the variance of 31·6 ascribable to error in the Analysis of Variance given later as applicable also to variations in the controls we obtain a value for "t" equal to $\frac{838}{\sqrt{31{\cdot}6\times6\times2}}$ =nearly 43—an exceptionally significant value. In view of this very definite result regarding the efficacy of sulphur dioxide as a nematocide,

statistical investigation of the figures in this Table was confined to the various treatment groups and a complete analysis of variance of these figures is given in Table 2. It will be seen from this that the total variance (V_9) is itself ascribable in varying degrees to eight different components :—V_1 the error variance ascribable to variations within treatment groups; V_2, V_3 and V_4, the variances due to concentration, humidity and time respectively; V_5, V_6 and V_7 due to interactions between humidity and concentration, concentration and time, and time and humidity respectively, as well as the triple interaction V_8 between concentration, humidity and time. The last column, variance-ratio in which all variances are related to V_1, the error variance, shows, on reference to the table of variance ratio for the appropriate number of degrees of freedom, that V_2, V_3, V_5 and V_6 are all significantly greater than V_1. Hence on further reference to the data of Table 1, conclusions can be drawn regarding the importance of the various factors involved in the reaction.

TABLE II.

Analysis of Variance on Figures in Table I.

Source.	Sum of Squares.	Degrees of Freedom.	Variance.	Variance ratio.
Error	1,896	60	31·6 (V_1)	·1
Conc.	530·4	2	265·2 (V_2)	8·4
Humidity ...	6,050·0	1	6,050·0 (V_3)	195·0
Time.	80·2	1	80·2 (V_4)	2·54
C.×H.	390·2	2	195·5 (V_5)	6·17
C.×T.	102·6	2	101·3 (V_6)	3·21
M.×T.	61·2	1	61·2 (V_7)	1·97
C.×M.×T. ...	114·75	2	57·35 (V_8)	1·82
Total ...	9,225·3	71	129·93 (V_9)	—

Concentration.—The fact that V_2 is significantly greater than V_1 indicates that for concentrations ranging from 1 :8 to 1 :30, the effect of this factor is significant. The total hatches being 152, 243 and 311 for concentrations of 1 : 8, 1 : 16 and 1 : 30 respectively and the critical difference for these being $2\sqrt{31\cdot6\times24\times2} = 77.8$, if the 5% level of significance is accepted, the conclusion may be arrived at that in general the 1 : 8 concentration is more effective than either of the other two but that 1 : 16 is not significantly more effective than 1 : 30.

Humidity.—The high value of the humidity variance is indicative of the very great importance of this factor in the treatment. The variance appropriate to humidity is the highest in the whole analysis being 22·9 times as great as the next highest—that for concentration and therefore significantly greater than any of them. This is borne out in Table 1, where comparison of moist totals with dry totals shows that under moist conditions only 23 larvae hatched out while under dry conditions the corresponding number was 583—a difference of 560 which is nearly 61 times the standard error of the totals. The importance of ensuring amply humid conditions cannot therefore be overstressed.

Time.—Comparison of time variance with error variance discloses a ratio $V_4/V_1 = 2{\cdot}54$, which for $n_1 = 1$ and $n_2 = 60$ is not significant. In other words the difference between ten and twenty minues' treatment is not significant in the absence of other factors.

Interaction of Concentration and Humidity.—The significance of V_5/V_1 indicates the presence of an interaction between concentration and humidity. This is rendered apparent if the appropriate totals are set out as follows :—

	1 : 8	1 : 16	1 : 30
moist	3	5	15
dry	149	238	296

In all cases the moist totals are less than the dry totals by a quantity greater than double their standard error ($2\sqrt{31{\cdot}6 \times 12 \times 2} = 55$) and the conclusion can be drawn that for all concentrations moist conditions are more effective than dry. On the other hand concentration appears to have a different effect under moist conditions from the corresponding effect under dry conditions. Under moist conditions the maximum difference between concentration totals is 12—far less than the critical difference. Under dry conditions, on the other hand all three concentration totals are significantly different from one another. In other words, under moist conditions concentration is not an important factor while under dry conditions it is. In all concentrations, moist conditions are more effective than dry.

Interaction of Concentration and Time.—The ratio V_6/V_1 being 3·21 the table of variance ratio shows it to be significant and accordingly necessitates a further study of the interaction. The appropriate totals are as follows :—

	1 : 8	1 : 16	1 : 30
10 minutes	90	151	150
20 minutes	62	92	161

The standard error of these totals being $\sqrt{31{\cdot}6 \times 12 \times 2} = 27{\cdot}5$, the critical difference will be 55·0. Hence it will be seen that for concentrations of 1 : 8 and 1 : 30 time is without significance but for 1 : 16 concentrations 20 minutes is significantly more effective than ten. Moreover by studying concentration totals it will be observed that for ten minute treatments a 1 : 8 concentration is better than either a 1 : 16 or a 1 : 30 but there is no significant difference between the two latter. When the treatment is prolonged for 20 minutes both 1 : 8 and 1 : 16 concentrations are more effective than 1 : 30 but there is no significant difference between the two former. These discrepancies in the interaction explain the lack of significance of time in general as a direct factor in the action of sulphur dioxide on the cysts.

Interaction of Humidity and Time.—The low value of V_7/V_1 indicates the lack of any significant interaction between humidity and time.

Triple Interaction of Humidity, Concentration and Time.—As in the case of the humidity-time interaction, the value of $V_8/V_1 = 1{\cdot}82$ indicates the lack of significance in this triple interaction.

Summarising the results of this analysis of variance, the conclusion is arrived at that humidity is the master factor at work, all other factors being subsidiary to it in their effect. Concentration appears to be of some significance but for the times of treatment studied the effect of time is insignificant except in so far as its effect is manifested as an interaction on concentration; as an interaction on humidity its effect is without significance.

In order to determine whether or not the very encouraging results recorded above were indicative of a true lethal effect on the part of the sulphur dioxide, additional cysts from each treatment group were stored for three months and then subjected to the action of root excretion. Strong confirmation that the effect was in fact lethal was furnished by the fact that whereas the controls exhibited active hatching, in no case did more than one or two larvae hatch out of the treated cysts over a period of three weeks, and it is problematic whether or not these latter were capable of infecting new plants. Their condition was to say the least

of it, semi-moribund and no active healthy movement was exhibited by any of them. It would thus seem that the full lethal effect of sulphur dioxide is not manifested for some time after exposure to the vapour—the immediate effect being to render the great majority of the larvae incapable of hatching out of the cysts; those that survive this action rapidly lose their ability to do so. It might well be that the larvae which are not killed immediately are so reduced in vitality that they become incapable of surviving for any length of time inside the cyst even when no longer exposed to the action of the chemical.

Action of Sulphur Dioxide on Adherent Cysts.

In determining the technique to be followed for this experiment it was considered that it should as far as possible be capable of imitation on a commercial scale. It was felt since the preliminary experiments suggested a delay in the manifestation of the full lethal effect, that treatment should be carried out under such conditions that a reasonable interval of time might elapse between the treatment and the planting out of the potatoes. Dr. M. T. Franklin suggested to the author that a suitable time for treatment would be when the potatoes were laid out in the chitting sheds. It might then be possible to devise an efficient method of fumigating the sheds prior to chitting, thus destroying the cysts. Any delayed action would then have the whole of the chitting period in which to manifest itself in full measure.

An experiment on the fumigation of the seed was therefore devised. The source of the sulphur dioxide was burning sulphur in the form of ordinary sulphur candles commonly used for bacterial fumigation. The candles used were designed for the fumigation of 1,000 cubic feet, while the room used for the fumigation had a cubic capacity of approximately 860 cubic feet, tests being carried out with one and two candles. The usual precautions attendant on fumigation were observed and immediately prior to the start of fumigation, the potatoes were well watered with the spray nozzle of a watering can. The walls and floor were also well watered and a can of water placed immediately over the burning candle in order to ensure a thoroughly wet atmosphere. The seed used was an ordinary sample of the variety Gladstone grown in the Institute's field at Gamlingay and known as a result of previous tests to carry an adherent infection in the form of eelworm cysts.

After 24 hours' fumigation the potatoes were removed and samples from each treatment lot scrubbed. The cysts removed by scrubbing

were recovered and stored for one month and then subjected to the action of root excretion. Cysts recovered from the untreated controls exhibited active hatching but those from both lots of fumigated tubers did not respond. In all only one larva was found to have hatched from the treated cysts and this larva was at the time of observation to all appearances dead, being shrunken and deformed and exhibiting no sign whatever of movement. The cysts were dissected and the larvae inside were without exception shrunken and bent and it is not considered that any of them survived the treatment.

The Action of Sulphur Dioxide on Potatoes.

Having established the value of sulphur dioxide as a nematocide, it now remained to determine whether or not it had any harmful effect on the potatoes. As the fumigation would normally be carried out prior to sprouting, it was necessary to determine whether or not it affected the sprouting power of the potatoes. It would also be interesting to see whether or not it had any effect on sprouts already formed. The fumigation experiment recorded in the last section was accordingly carried out on sprouted tubers, partly with the object of making the test more stringent and also with the object of investigating both effects in one experiment. The treated seed was therefore divided into a number of samples.

One sample was merely stored with its chits attached. On removal after fumigation the chits appeared to be in quite healthy condition but after two days had elapsed, all the chits already formed had dried up, shrunk and died. This effect was equally apparent whether fumigation had been carried on with one or two candles. Chits were removed from some of the potatoes immediately after fumigation, treated with "Auxan" and planted in damp sand. It was found that whereas chits from untreated potatoes rooted freely and developed reasonably well, those from the fumigated seed showed a very high mortality which was very much more marked in the case of those fumigated with two candles. It is true that some of the fumigated chits survived but even so their progress was vastly inferior to that of the untreated controls.

In order to investigate the effect of the fumigation on the subsequent chitting power, further samples were taken, the chits already formed removed and the tubers put out to develop new ones. Casual examination revealed no difference between the controls and the fumigated seed, the sprouts being about equal in number in all three classes and

apparently equally vigorous and healthy. In order to carry out a more detailed and sensitive test twenty tubers selected at random from each group were taken and the number of chits on each counted. The results are set out in Table 3 and an Analysis of Variance on the figures is given in Table 4. It will be seen that the mean number of chits per tuber is 6·35 for controls, 5·2 for one candle treatment and 4·5 for two candle treatment. The variance ascribable to random errors within series (V_1) being 7·87 and that between series (V_2) being 17·4, the ratio V_1/V_2 is 2·20 : 1, which for $n_1=2$ and $n_2=57$ is not significant.

TABLE III.

The Effect of Fumigation on the chitting Power of Potatoes.

	Frequency of occurrence of numbers of chits per tuber.														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean.
Controls ...	—	1	1	1	2	2	2	5	4	—	1	—	—	—	1	6·35
1 Candle ...	2	—	—	2	4	4	2	2	1	2	1	—	—	—	—	5·20
2 Candles	2	1	1	4	3	2	3	1	—	2	1	—	—	—	—	4·50

TABLE IV.

Analysis of Variance of figures in Table III.

Source.	Sum of Squares.	Degrees of Freedom.	Variance.	Variance ratio.
Error	448·75	57	7·87	1
Between series	34·9	2	17·45	2·20
Total	483·65	59	9·0	—

The experiment therefore failed to detect any significant difference between the chitting powers of treated and untreated tubers, and it is considered justifiable to conclude that sulphur dioxide fumigation has little if any effect on the chitting powers of the potato.

A further and final experiment was performed in which the tubers immediately after treatment were planted in pots alongside untreated controls. Twelve tubers were planted in each series and the sprouts were not detached before planting. The untreated controls broke surface the fourth week after planting. The treated seed did not show through until the sixth week in the case of the one candle group, while the two candle group did not show through until the seventh week after planting. All twelve of the controls showed through, while among the

one candle group there was one failure, and among the two candle group there were four failures. It is considered that these results tally to a very large extent with the previous results. A possible reason for the slowness of the treated seed in appearing is that the sprouts already formed when the tubers were exposed to the fumigation died, these potatoes consequently being at a disadvantage compared with the controls the sprouts on which were presumably healthy and virile. Had both lots been sprouted subsequently to the fumigation it is safe to assume that this time lag would no longer be apparent. The failures in this experiment probably correspond to those tubers in Table 2 which did not develop new sprouts.

Summarising the results of the experiments on the fumigation of the potatoes, the conclusion may be arrived at that sulphur dioxide used under conditions appropriate to bacterial fumigation has little harmful effect on the chitting power of the potatoes. It is however lethal to the chits already formed and the fumigation should therefore be carried out prior to chitting.

Summary and Conclusions.

1. "In vitro" experiments show that sulphur dioxide is very lethal to cysts of the potato eelworm. Its effect is very much more potent under moist conditions than under dry. The other factors, concentration and time are secondary in their action as compared with humidity.

2. Fumigation of seed potatoes for 24 hours under conditions appropriate to bacterial fumigation results in the complete destruction of the cysts adherent to them.

3. The sulphur dioxide fumigation is without any significant effect on the tubers provided it is carried out previous to chitting. It has little if any effect on the chitting power of the potato but is lethal to any chits already formed.

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The Degree of *Heterodera* Infectivity of Soil and its Determination.

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INTRODUCTION.

UP to the present time the measurement of the infectivity of soil with eelworm has been accomplished by determining the number of cysts present in a given mass of soil. Earlier workers (e.g. Morgan, 1925) shook up the soil with water in a litre flask and counted the cysts present in the float without seeking to determine by statistical analysis the errors attendant on the method. The author in a recent paper (Fenwick 1940) describes a new method of enumerating cysts per mass of soil using a special apparatus devised for the purpose; the figures obtained by this apparatus were analysed statistically and a measure of the accuracy of the method was arrived at. No attention was paid to the degree of infectivity of individual cysts. Subsequent work convinced the author that this latter quantity varied very greatly for cysts recovered from various soils. It was frequently found, for instance, that of a sample of cysts taken from one soil, only about 15% contained eggs, while the egg content of this 15% was only 20–30 eggs per cyst. In other cases about 90% of the cysts contained eggs, while the egg content of this 90% was often 200–300 eggs per cyst. Moreover, frequently eggs present in the cysts were found on examination to be empty, there being no larvae present. It was felt that this great variability in the " quality " of cysts was sufficient to nullify any value which might be placed on cyst counts as a criterion of soil infectivity and the author accordingly decided to investigate the possibility of finding some more fundamental measure.

It would perhaps be advantageous at this stage to decide exactly what is the problem to be faced. In the writer's opinion it is this—to ascertain the level of eelworm infection in any given soil—in other words to determine the number of organisms present per mass of soil which under theoretically ideal conditions might be capable of entering the roots of susceptible plants placed in the soil. Consider the history of Heterodera cysts in soil. Each contains a variable number of eggs which may or may not contain a larva. These larvae, under the stimulus of root excretion, can hatch out of the egg, emerge from the cyst and ultimately penetrate a rootlet, causing a new infection. It is thus readily seen that the key to the problem is the larva, since this is the true infective agent. The cyst, for the purpose of determining infectivity, is merely a container for an unknown number of eggs, each of which might or might not contain a larva. Thus the problem resolves itself into determining the number of larvae present, each of which is potentially capable of entering a fresh host and setting up a new infection therein. It is considered that this concept represents a more fundamental approach to the problem than does the older idea of cyst content and the experiments to be described represent an attempt to measure soil infectivity in terms of this concept, i.e. to determine the number of larvae present per mass of soil. In deciding the line of investigation to be followed numerous avenues of approach were considered and rejected for various reasons, since it was felt that the final technique had of necessity to conform to certain conditions. In the first place, however complicated or otherwise the theory underlying it might be, the final method had to be simple and easy to apply. In addition, it was imperative that the results obtained should be constant in the hands of different operators and for varying seasons of year. In fact what was needed was a simple, practical method requiring the minimum degree of manipulative skill in its application, and yielding consistent results under a variety of conditions, for determining to a known degree of accuracy the number of Heterodera larvae present within the cysts in a known mass of any given soil.

For obvious reasons the biological method of hatching out the larvae in root excretion was unsatisfactory. In the first place the rate of hatching in this medium is governed to a very large extent by the time of year, being very rapid in the early summer months and slow in the winter. Root excretion itself is very indeterminate in its action, some

samples causing very active hatching and others very retarded hatching. Hence it is obvious that any method based on hatching of larvae in this substance must yield very variable results.

Methods based on the dissection of recovered cysts proved unduly tedious and time consuming in view of the large number of cysts which it was found necessary to examine in order to obtain a representative cross section of the cyst population.

Yet another method of investigation suggested was to grow test plants in the infected soil and at the end of a certain time to determine the number of developing organisms in the plant tissue. Whilst this method, being based directly on the infectivity of the soil to the plant itself was considered to be the most fundamental, it suffered from the disadvantage that it involved too many variable and unknown factors, at the same time being thoroughly impracticable from the point of view of the time required in order to obtain a result for any given soil.

Yet another method arrived at—the one finally adopted as a basis of investigation—was to hatch out the larvae by chemical means. Such a method had the advantage of being capable of careful and accurate control. The action being chemical in nature was moreover unlikely to be dependent on factors like time of year, etc. The experiments herein described are accordingly concerned with the developments of a technique for enumerating larvae obtained from the cysts by chemical hatching.

Procedure.

In searching for a chemical agent which would cause the liberation of Heterodera larvae from cysts, attention was paid to the fact that hypochlorite solutions had a solvent effect on the cysts (Smedley 1936). Smedley found that if cysts were immersed in solutions of hypochlorite one of two things would occur according to the nature of the metallic ion present. If the metal were sodium then the cyst ruptured and finally dissolved, but the action was curious in that the egg shells were not attacked despite the fact that the larvae inside were dissolved, so that the end result was the liberation of large numbers of empty egg shells. In the presence of the calcium ion, however, a different action occurred—the cyst was ruptured and eventually dissolved, the egg-shell itself was attacked but the solvent action of the larvae was far less, so that in this case the net result was the liberation of the larvae which, however, on prolonged exposure to the solution dissolved. These findings by Smedley are, generally speaking, in accordance with

the previous findings of Molz (1930, 1932). This solvent action of hypochlorites also forms an interesting parallel of the findings of Lapage (1933, 1935) regarding the action of sodium hypochlorite in the form of the proprietary compound " Milton " on Trichostrongylid larvae and also the author's findings (Fenwick 1939) regarding the action of the same substance on infective eggs of *Ascaris lumbricoides var. suum*.

It was accordingly decided that the first step to be taken was a detailed investigation into the action of hypochlorites on eelworm cysts. Smedley's results regarding the different effects produced by sodium and calcium hypochlorite respectively were first confirmed, and experiments henceforward were confined to the calcium salt. At this stage it would be convenient to utter a word of caution. Calcium hypochlorite may be obtained in a variety of forms, the commonest being the ordinary domestic bleaching powder as well as numerous proprietary compounds made by different chemical firms. In no case is the product pure calcium hypochlorite. In practice samples invariably consist of calcium hypochlorite in the presence of calcium hydroxide, calcium chloride and frequently calcium carbonate. The proportions of each vary over very wide limits and consequently it is futile to expect consistent results from solutions containing " so many grammes of bleaching powder per litre." It is imperative that all experiments be based on the available chlorine content of the final solution as determined by chemical analysis. Practical details for this determination are given in a later section of the paper dealing with the finally established technique. Moreover it is important that all solutions should be made up with cold freshly boiled distilled water as otherwise the carbon dioxide present results in the precipitation of calcium as calcium carbonate. These precautions were stringently observed in all the experiments described in this paper.

Action of Calcium Hypochlorite Solutions on Heterodera Cysts.

A series of experiments was conducted on the effect of different strengths of calcium hypochlorite solutions on the cysts and the first effect observed was that the action of the solutions on thoroughly dry cysts was very slow but that if the cysts were soaked for twenty-four hours, even though they still floated, the action of a given strength solution was much more rapid and consequently all cysts were soaked for twenty-four hours before experiment. Different strengths of solution were used varying from 3% to 0·1% available chlorine. It was found that the stronger the solution the more rapid was its action both in liberating

the larvae and also in dissolving them after liberation. Attempts to devise a modified Baerman apparatus for hatching out the larvae in a fairly strong hypochlorite solution and transferring them to water after hatching were unsuccessful and were very soon abandoned, it being considered that the technical difficulties encountered were too great. It was therefore decided that a more profitable method of approach was to determine the rate of solubility of the larvae in different strengths of hypochlorite and also the rate of hatching in the same strength and apply corrections to the observed number of larvae liberated to allow for those which had been dissolved. Investigation along these lines disclosed the fact that in solutions containing 1% available chlorine hatching rates were very high, hatching being more or less complete in three hours, while the number of larvae dissolved in this time was very small. Hence an experiment was devised in two parts.

For the first part single cysts were placed in watch-glasses of bleaching powder solution containing 1% available chlorine and the number of larvae present at the end of certain times noted. Fifty cysts selected at random from samples taken from a variety of soils were thus treated and the results are set out in Table I. It will be seen that after three hours, in all cases except one (cyst No. 29) nearly all the larvae were liberated, and it was concluded from this that in any batch of cysts the number of larvae not liberated after three hours would be negligible compared with the number set free.

The second part of the experiment consisted of putting up larvae in batches of one hundred each in hypochlorite solutions containing 1% available chlorine, and counting the number of larvae which had resisted solution at the end of certain times. It was found distinctly advantageous in this and in the previous part of the experiment to cover the fluid in the watch-glasses with a thin layer of xylene, which sealed the fluid from the air thus eliminating the scum which invariably forms on the surface when bleaching powder solutions are exposed to the air. The results are set out in Table II, and it will be seen that after four hours, of 1,200 larvae exposed only 12 were dissolved—in other words 1%. A statistical analysis of the figures in Table II yields interesting results. Since at the end of four hours 1% of the larvae are dissolved and 99% are not, if p represents the probability of a larva surviving and q the probability of its not surviving solution, then $p = 0{\cdot}99$ and $q = {\cdot}01$. In tests with a hundred larvae, the proportion of tests giving 100, 99, 98, etc., larvae undissolved will be given by the

TABLE I.

The Rate of Hatching of single Cysts exposed to Bleaching Powder Solutions containing 1% available Chlorine.

Cyst No.	Number of Larvae Present.						Larvae left in cyst.
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	
1	153	178	181	182	182	182	0
2	167	193	196	195	195	195	0
3	73	79	81	81	81	80	0
4	217	240	240	240	241	241	0
5	23	36	37	38	38	38	0
6	124	157	158	157	158	158	1
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
9	234	243	246	244	244	244	0
10	193	207	208	211	212	210	3
11	124	139	138	139	138	138	0
12	0	0	0	0	0	0	2
13	3	6	6	6	6	6	0
14	21	43	43	44	43	43	0
15	219	263	263	264	263	263	0
16	241	298	299	299	298	299	0
17	147	218	218	218	219	216	0
18	298	357	369	370	370	370	0
19	12	17	17	18	18	18	0
20	93	98	112	113	113	113	0
21	167	194	195	195	195	194	0
22	98	106	105	105	105	104	0
23	108	123	125	125	125	123	0
24	0	0	0	0	0	0	0
25	9	13	13	13	14	14	0
26	197	221	223	223	225	225	0
27	9	12	12	12	12	12	0
28	127	137	138	138	136	137	0
29	0	0	0	0	0	0	132
30	232	233	234	234	233	233	1
31	176	203	208	208	208	209	0
32	135	186	188	189	189	191	0
33	132	138	143	144	144	143	0
34	92	97	107	109	110	110	0
35	193	224	229	228	228	228	0
36	134	147	154	154	154	154	0
37	37	39	46	46	46	45	0
38	83	93	94	94	96	94	0
39	283	301	327	326	326	326	0
40	191	217	224	224	224	224	0
41	0	0	0	0	0	0	0
42	3	4	4	5	5	5	0
43	7	9	12	12	12	12	0
44	137	154	156	156	157	156	0
45	0	0	0	0	0	0	1
46	234	273	276	276	275	275	0
47	165	179	179	180	181	181	0
48	117	132	134	135	134	134	0
49	193	246	246	247	247	247	0
50	204	212	223	226	226	226	0

expanded terms of the binomial $(p+q)^n$ where $p=0{\cdot}99$, $q={\cdot}01$ and $n=100$. The first few terms of this expansion when calculated give an expected frequency of occurrence as follows :—

x	100	99	98	97	96
F^1	4·368	4·44	2·22	·756	·180
F^2	6	2	3	0	1

where x = number of larvae surviving and F^1 is the expected frequency of occurrence of any particular value of x. F^2 is the actual observed frequency of x. Comparison of F^1 and F^2 by the χ^2 test disclosed close agreement. Hence we are justified in concluding that the results shown in Table II fall into a binomial distribution. Now

TABLE II.

Table showing the Survival of Larvae of *H. schachtii* exposed in Batches of 50 to the action of a Bleaching Powder Solution containing 1% available Chlorine.

Time.	Bath Number.											
	1	2	3	4	5	6	7	8	9	10	11	12
Start ...	100	100	100	100	100	100	100	100	100	100	100	100
½ hr. ...	100	100	100	100	100	100	—	—	—	—	—	—
1 ,, ...	100	100	100	100	100	100	—	—	—	—	—	—
1½ hrs....	100	100	100	100	100	100	—	—	—	—	—	—
2 ,, ...	100	98	100	99	100	100	—	—	—	—	—	—
3 ,, ...	100	98	100	99	100	100	—	—	—	—	—	—
4 ,, ...	100	98	98	99	100	100	100	100	96	100	98	99
6 ,, ...	100	97	96	99	100	100	100	100	96	97	98	98
8 ,, ...	—	—	—	—	—	—	—	—	—	—	—	—
10 ,, ...	98	95	96	97	96	100	—	—	—	—	—	—
16 ,, ...	—	—	—	—	—	—	97	98	95	97	98	94
20 ,, ...	—	—	—	—	—	—	96	94	90	96	92	91
24 ,, ...	94	91	92	89	91	95	94	90	87	89	90	87
30 ,, ...	90	89	90	89	86	91	—	—	—	—	—	—
40 ,, ...	—	—	—	—	—	—	84	83	78	86	90	85
48 ,, ...	81	78	84	80	79	87	80	76	79	82	80	79

for a binomial distribution the standard deviation of the probability of an event occurring (in this case of a larva surviving) (p) is $\sqrt{\frac{pq}{n}}$, n for the whole experiment being 1,200. Hence we arrive at the conclusion that the probability of the larva surviving is 0·99 with a standard deviation of $\sqrt{\frac{\cdot 99 \times \cdot 01}{1200}}$ ·0028. It is therefore quite safe to assume that at the end of four hours the number of larvae dissolved is very unlikely to exceed to any great extent 1% of the number present in the

free state at the commencement of this time. Moreover it will be seen that at the end of four hours hatching very few larvae will have existed in the free state for the whole of this time, and therefore the loss of larvae will in all probability be less than 1%, and it is considered that this very small loss can be safely neglected for the purposes of measuring infectivity. Hence it may be said that the number of larvae liberated from cysts at the end of four hours in a bleaching powder solution containing 1% available chlorine is a close estimate of the number originally present in the cysts and the sole problem remaining is the enumeration of these larvae.

ENUMERATION OF LARVAE LIBERATED BY HYPOCHLORITE.

It is obvious that since half-pound samples of soil frequently contain hundreds of cysts, each of which may contain hundreds of larvae, it is quite impracticable to count all the larvae liberated from all the cysts. Hence it was necessary to resort to some dilution method. Peters and J. W. G. Leiper (1940) working on infections of *Haemonchus contortus* in lambs, showed that a test of egg counts made by a modification of the McMaster dilution technique conformed approximately with the Poisson distribution even though their counts (275 in all) involved eleven series of 25 counts each, each series having a different mean. These findings led the writer to consider whether or not the same would be true of counts made by a similar technique with the larvae liberated from eelworm cysts by means of hypochlorite and an experiment was performed to test out this possibility.

To this end a 30 lb. sample of soil was thoroughly mixed by coning and quartering and ten half-pound samples were floated up in the apparatus described in the author's previous paper. The ten floats thus obtained were soaked in water for twenty-four hours and then subjected to the action of a bleaching powder solution containing 1% available chlorine for four hours. Each treated float was washed through a hundred mesh sieve to remove large particles of débris and the washings centrifuged, the centrifuged mass being washed and made up to 500 ccs. with water. The suspensions thus obtained were agitated by up and down movements of a plunger as described by Peters and Leiper; samples were withdrawn and run into the cells of a modified McMaster slide and the larvae present per 0·15 ccs. counted. Ten such counts were performed on each suspension.

The McMaster slide used in this experiment differed from the standard model only in that the etched square instead of being on the lower

surface of the top glass was in this case marked on the top surface of the lower glass. This modification was necessitated by the fact that the larval suspensions being made up in water, the larvae in the slide sank and in a standard slide the etchings and the larvae would lie in different focussing planes. Experiments subsequently performed showed that identical results were obtained if the suspensions were made up in brine and a standard slide used.

TABLE III.

Counts obtained from ten Samples each of 0·15 cc. of Suspension taken from each of ten Half Pound Samples of Soil.

Count No.	Soil Sample Numbers.									
	1	2	3	4	5	6	7	8	9	10
1 ...	18	18	7	8	11	7	19	11	8	13
2 ...	10	10	14	5	10	8	7	12	6	14
3 ...	9	11	9	6	18	7	9	8	12	17
4 ...	12	18	10	7	10	14	10	11	11	12
5 ...	14	14	8	3	6	9	13	10	7	11
6 ...	13	10	12	16	17	12	8	7	6	13
7 ...	8	13	9	7	8	6	11	13	13	6
8 ...	16	9	12	15	11	15	14	12	14	12
9 ...	10	14	8	18	9	9	8	15	10	12
10 ...	8	7	13	8	7	17	10	13	7	10
Mean ...	11·8	12·4	10·2	9·3	10·7	10·4	10·9	11·2	9·4	12·0
Total ...	118	124	102	93	107	104	109	112	94	120

Grand Total of all values of x ... 1,083

The results of this experiment are set out in Table III. It will be seen that they consist of ten series of ten counts, each with wide individual variations between counts and also between means. It now remained to determine whether these differences between means were statistically significant or whether they were merely the results of random variations in 0·15 cc. samples and an analysis of variance was therefore performed to obtain information on this point. If x represents individual values, G group totals and x the grand total, then $\sum x^2 = 12,837$, $\sum G^2 = 118,419$ $x^2 = 1,172,889$ and M the grand mean = 10·83. An analysis of variance in tabular form is shown in Table IV. Reference to tables of variance-ratio for values of n_1 and n_2 of 9 and 90 respectively shows that accepting 5% levels of significance the variance ratio must exceed 1·83 while for a 20% level is must exceed 1·35. Therefore the value of 1·136 shown in Table IV indicates that the differences between the means are not significant and it can be assumed that all

the individual values of x fall into one big series with a common mean.

It will be observed that the value of 11·06 for the variance in Table IV is very close in value to the mean. This very close agreement

TABLE IV.

Analysis of Variance on Figures in Table III.

Source.	Sum of Squares.	Degrees of Freedom.	Variance	Variance Ratio.
Group	$\frac{\Sigma G^2}{10} - \frac{X^2}{100}$ $=11,841\cdot9-11,728\cdot9$ $=113$	9	12·56	1·136
Error	$\frac{\Sigma x^2}{1} - \frac{\Sigma G^2}{10}$ $=12,837-11,841\cdot9$ $=995\cdot1$	90	11·06	1
Total	$\frac{\Sigma x^2}{1} - \frac{X^2}{100}$ $=12,837\cdot0-11,728\cdot9$ $=1,108\cdot1$	99	11·19	—

TABLE V.

Table for a χ^2 Test on values of x in Table III.

x	O	E	$\frac{(O-E)^2}{E}$
7	8	8·1502	0·00276
7	11	6·9367	2·39300
8	12	9·3646	0·74143
9	7	11·2375	1·59752
10	12	12·1365	0·001535
11	8	11·9159	1·26350
12	9	10·7213	0·2771
13	10	8·909	0·1336
14	9	6·873	0·6582
14	15	13·7985	1·0462
	$\frac{(O-E)^2}{E}=\chi^2=8\cdot114853$		

between the mean and the variance is a characteristic of the Poisson series and a test was performed to determine whether or not the distribution of counts in Table III agreed with the expected Poisson distribution for a mean of 10·83. Table V shows in tabular form the

expected and observed frequencies of occurrence of different values of x, when O is the observed frequency, E the expected frequency as determined by reference to the table of $\frac{e^{-m}m^x}{\lfloor x.}$ for a mean of 10·83. The total of the last column, i.e. the summation of $(O-E)^2/E$ is χ^2 and in this case amounts to 8·114853 and reference to the table of χ^2 indicates a probability of nearly 0·50 of obtaining a χ^2 value exceeding this figure. The conclusion was therefore drawn that all the values of x for all ten soil samples fall into one single Poisson series with a single common mean. This coupled with the fact that the differences between the ten means are not significant indicates that it is unnecessary to examine more than one half-pound sample from a mass of soil brought into the laboratory.

TABLE VI.

Numbers of Larvae to be counted (N) in order to obtain known Degrees of Accuracy.

Percentage Accuracy (y)	No. of Larvae to be counted (N)
1%	40,000
5%	1,600
10%	400
20%	100
50%	16

There is a further implication to this finding that the distribution of counts fall into a Poisson series. The mean of such a series is numerically equal to the variance so that if x represents individual values of x, and $\bar{x}$ the mean, then variance $=\bar{x}$ and the standard deviation is $\sqrt{\bar{x}}$. For n counts performed the standard error would be $\sqrt{\frac{\bar{x}}{n}}$ and 95% of means obtained would fall between $\bar{x} \pm 2\sqrt{\frac{\bar{x}}{n}}$. Suppose it is desired that this error should not exceed y% of the mean, then $2\sqrt{\frac{\bar{x}}{n}} = \frac{y}{100}\bar{x}$ and $\frac{4\bar{x}}{n} = \frac{y^2\,\bar{x}^2}{10{,}000}$—in other words $40{,}000 = y^2nx$, but nx is the total number of larvae counted $=N$, say; then $40{,}000 = Ny^2$. So that expressed simply the accuracy of the determination expressed as the square of a percentage of the mean obtained is equal to 40,000 divided

by the total number of larvae counted. Table VI shows the total number of larvae to be counted in order to obtain different degrees of accuracy. It is considered that ± 10% is a sufficiently high standard of accuracy, and it is accordingly suggested that 0·15 cc. samples be examined until 400 larvae have been counted when the accuracy of the mean will be ±10% in 95% of cases.

Tests on Different Types of Soil.—In order to determine whether the conclusion previously arrived at that it was unnecessary to examine more than one half-pound sample of soil from a mass was true for different types of soil, four soils differing widely in their texture, composition and cyst content were examined. The technique used for this test was exactly similar to that already described except that the suspensions were made up to 100 ccs. instead of 500 ccs. as it was found that with most soils 0·15 cc. samples taken from 500 ccs. of suspension gave such low counts that an unduly large number of samples had to be examined in order that a reasonable number of larvae should be counted. In each case three half-pound samples from a five-pound mass of soil was floated and ten lots of 0·15 cc. of suspension from each examined. The results are set out in Table VII. An analysis of variance was performed on the data and is given in Table VIII. No explanation is given for the very low sample variance as compared with the error variance and which presumably can only be explained in terms of pure chance, but it very definitely indicates that the differences between the means of half-pound samples from any one soil are not significant, and the conclusion already drawn that it is unnecessary to examine more than one half-pound sample is amply confirmed.

The calculation of the infectivity of soil in terms of the number of larvae present per unit mass is simple. The mean value of x for 0·15 cc. of suspension is taken and from this the number present in the whole volume of suspension is calculated. This doubled gives the number present per pound of soil. The standard error of the determination is of course known, being determined solely by the number of larvae counted. A result is thus obtained for the number of larvae present and potentially capable of infecting susceptible plants grown in the soil. This has been worked out for the soils used in evolving the technique described in this paper and the figures obtained are set out in Table IX. In this table the soil from Ayrshire is the one to which the figures in Table III refer. The other soils are those referred to in Table VII. The cyst counts for these soils in cysts per pound are also given as arrived at by the author's differential flotation technique

TABLE VII.

Larvae present in 0·15 cc. Samples of Different Types of Soil.

Count Number.	Ervine soil.			Maiden soil.			Potton soil.			Winches soil.		
	1	2	3	1	2	3	1	2	3	1	2	3
1 ...	12	13	17	51	58	57	59	70	61	8	9	4
2 ...	17	14	14	60	55	58	62	59	69	4	6	3
3 ...	14	15	11	57	57	60	71	66	63	5	7	9
4 ...	13	12	13	53	59	59	63	72	56	4	3	6
5 ...	15	15	14	63	51	57	74	67	69	6	6	6
6 ...	21	17	9	58	57	54	70	64	65	3	1	7
7 ...	10	12	15	59	56	54	76	63	66	5	2	4
8 ...	15	14	15	55	62	58	61	66	72	9	8	8
9 ...	12	13	16	53	58	53	64	61	67	6	7	10
10 ...	15	10	12	56	56	55	63	68	68	7	1	4

TABLE VIII.

Analysis of Variance on Figures in Table VII.

Source.	Sum of Squares.	Degrees of Freedom.	Variance.	Variance Ratio.
Soils	81,990	3	27,330	14,489
Between Soil Samples ...	15	8	1·9	1
Error	1,223	108	11·3	5·4

TABLE IX.

Larval Content of Soils used together with their corresponding Cyst Counts.

Soil	Mean of 0·15 cc. Counts.	Total No. of Larvae counted.	Percentage Error.	Volume of Suspension.	Larval Content per Lb.	Cyst Content per Lb.
Ayreshire	10·83	1,083	6·3%	500 ccs.	72,000 ±4,536	1,776±5·6
Ervine ...	13·8	415	10%	100 ccs.	18,400 ±1,840	84·8±5·2
Maiden ...	56·7	1,701	5%	100 ccs.	76,266 ±3,813	3,843±7·3
Potton ...	65·8	1,975	5%	100 ccs.	87,733 ±4,386	538±7·5
Winches	5·6	168	5%	100 ccs.	7,466·7 ±4,386	233·6±4·2

(Fenwick 1940). A study of these figures for the cyst content and the potential larval content shows the very definite lack of correlation between the two quantities and offers striking evidence of the inadequacy of cyst counts alone as a means of measuring infectivity, e.g. if we compare Irvine soil with soil from Winches' Farm (St. Albans), the latter appears to be far more heavily infected than the former if cyst counts are taken as a criterion, but using the larval technique it is found that Winches' Farm soil contains far fewer larvae than does Irvine soil, due to the fact that whereas Irvine cysts are reasonably full, Winches' Farm cysts are nearly all empty.

FINAL DESCRIPTION OF TECHNIQUE WITH PRECAUTIONS TO BE TAKEN IN ITS APPLICATION.

It has been mentioned earlier on that the most important precaution to be observed is the making up of the hypochlorite. For the experiments described the purest obtainable calcium hypochlorite supplied by British Drug Houses, Ltd., was used since it proved the most constant in strength. It was found that if a 5% solution of this was made up with cold freshly boiled distilled water, the resulting solution after filtration had a strength of approximately 1% available chlorine. The strength of this solution can be determined rapidly and with sufficient accuracy as follows. Take 10 ccs. of the filtered solution, acidify with pure hydrochloric acid and add 10 ccs. of 10% potassium iodide solution. Run in from a burette, decinormal sodium thiosulphate solution (24·8 gms. $Na_2S_2O_3 \cdot 5H_2O$ per litre) until the brown coloration formed on adding the iodide becomes pale yellow. A few drops of cold freshly boiled starch solution should then be added and more thiosulphate run in until the blue coloration formed just disappears. The strength of the thiosulphate solution can then be calculated on the basis that if it is 1% available chlorine, then using 10 ccs. to start with, it should require 28·5 ccs. of thiosulphate. If it is made overstrength to start with, it is then a simple matter to adjust its strength to 1% by dilution. In practice it was found that the solution could vary between 0·8 and 1·2% available chlorine without any harmful effect, moreover provided that the solution was stored in a well stoppered bottle, it retained its strength reasonably well for about 14 days.

The several stages in the technique are now as follows :—(1) Recover the float obtained from a half-pound sample of soil by the method described in the author's previous paper or by any other method

giving a comparable degree of accuracy and allow the float to soak for 24 hours.

(2) Treat the float with the hypochlorite solution for four hours, wash through a 100 or 120 mesh sieve and centrifuge the washings. The centrifuged mass should then be agitated to form a suspension and made up to 100 ccs. with equal parts of saturated brine and water.

(3) After careful mixing with up and down movements of a plunger in a measuring cylinder, samples should be withdrawn and the number of larvae present per 0·15 cc. counted in a standard McMaster slide. Successive samples should be examined until a total of 400 larvae are counted. The means of these counts will then be accurate to $\pm 10\%$ in 95% of cases and the larvae potentially present per pound of soil can be determined by calculation.

N.B.—It is important that the counts be performed immediately after making up the suspension otherwise the brine being hypertonic to the larvae will in time cause shrinkage of the larvae, rendering them quite unrecognisable.

Viability of the Larvae.—It will be noted that in the description of technique given, no account is taken of whether the larvae originally present in the cysts are alive and viable or not. In the writer's opinion this is not very satisfactory and several attempts were made to determine what proportion of the larvae were alive. Attempts to this end were handicapped by the lack of any reliable and convenient method of distinguishing between living and dead larvae. A technique was evolved which rendered possible the liberation of large numbers of larvae from the cysts in a living condition but the application to these of Boyd's Iodine Technique (Boyd, 1941) resulted in very indefinite and inconsistent results. Further research is being undertaken on the viability of the larvae, and it is hoped finally to evolve a technique for determining what proportion of the larvae present within the cysts are viable and thus obtain a true measure of the infectivity of the soil. In the meantime it is felt that even in its present form, the technique described gives a more fundamental measure of soil infectivity than does any method based on cyst counts.

Differentiation of Different Species of Larvae Present—The technique described does not seek to differentiate the different species of Heterodera larvae which might be present nor to determine what proportion of each species there is present. It is obviously of great importance

that this problem of different strains should be solved, especially so in view of Franklin's findings (Franklin, 1940) that the different strains in fact constitute completely different species, with very definite host-parasite specificity. It is hoped as a result of research already in progress, carried out jointly with Dr. Franklin, that a solution to this problem will shortly be found.

SUMMARY AND CONCLUSIONS.

(1) A technique for measuring the infectivity of soil with *Heterodera schachtii* in terms of the number of larvae potentially capable of infecting plants per pound of soil is described.

(2) The liberation of the larvae is accomplished by subjecting the cysts recovered from one half-pound sample of soil to a given strength of calcium hypochlorite solution for four hours. The loss of larvae due to solution during this time was found to be negligible.

(3) It was shown that if the larvae liberated were made up to a suspension, then counts made on 0·15 cc. samples of this suspension fall into a Poisson distribution and hence the accuracy of the final determination is dependent only on the number of larvae counted.

(4) It was found that provided the soil to be examined was thoroughly mixed that it was unnecessary to examine more than one half-pound sample as there was no significant difference between the means from different samples.

(5) Figures of cyst counts tabulated alongside available larvae show the utter inadequacy of cyst counts as a measure of infectivity.

(6) No attempt is made in this paper to determine how many of the larvae are alive and viable, further research into this matter being contemplated.

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Identification of *Heterodera* Species by Larval Length. Technique for Estimating the Constants determining the Length Variations Within a given Species.

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Introduction.

The destruction in the soil of the cysts of the several cyst-forming species of *Heterodera* which cause serious damage to agricultural crops has so far proved to be impracticable, and the only measures which can yet be recommended for the control of these parasites are good husbandry and the cultivation of immune crops. Good husbandry is a matter for the agriculturist, while the specialist in helminthology must provide exact information on the host range of the eelworms and the identification of the species concerned.

The problem of host range has been very much complicated in the past by the fact that it was not always realised that more than one species of *Heterodera* was involved. For example, host lists of the " sugar beet eelworm " (*H. schachtii*) have been published, including oats, potatoes and peas. While it was well known that the parasites attacking certain plants, such as potatoes and cereals, appeared to be restricted to these hosts and their near relatives, this was attributed to the development of biological races, which were all assumed to belong to the one species and in which the host specialisation might break down at any time. With the splitting of *H. schachtii* into four or five species (Franklin 1940), each with its own host range, there appears to be more ground for assuming the host range of each species to be fairly rigid, but a great deal of further investigation is required in order to determine exactly the limits of these host ranges.

The second part of the problem, that of the identification of the parasite, may be tackled by the attempted infection of all its possible host plants, but this is a slow method of the hit-or-miss variety. What is required is a means of identifying, by inspection, the eelworm cysts in a soil sample. Goffart (1934) gives a scheme for distinguishing the beet, oat and potato " strains " of *H. schachtii* (as they were then considered to be) from the characters of their cysts and cyst contents.

The identification rests on the colour, shape and length/breadth ratio of the cysts, and on the shape of the eggs and length of the larvae. The rounded cysts of *H. rostochiensis* and the ovoid ones of *H. punctata* are easy enough to identify, but since there are now recognised at least four species of *Heterodera* having " lemon-shaped " cysts, including the very commonly occurring species parasitising wild clovers, the differences given by Goffart are insufficient for identification.

The possibility of being able to identify a cyst by its size, colour and shape appears remote if one examines a number of cysts from the same host plant. There is always a great deal of variation in all these characters. It is true that Goffart (1930) found significant differences in the length/breadth ratios between cysts of the sugar beet and oat " strains," and of the sugar beet and potato " strains," but no significant differences between the length/breadth ratios of cysts derived from three other hosts of the sugar beet eelworm; i.e. the cyst ratio did not vary within the species of *Heterodera* attacking sugar beet and allied hosts, but it did vary significantly between some of the species which Goffart compared. To determine the species of *Heterodera* by this means, however, requires a fairly large number of cysts, whereas the ideal is to be able to identify individual cysts. In the apparent absence of morphological differences in the cyst walls, all that remains is to examine the larvae within the cyst, and the only character of the larva which has shown any measureable variation which might be of use in identification is the length.

A preliminary consideration of the figures given in the literature for the length of the larvae of *Heterodera* species leaves one with the impression that this is such a variable character that it cannot have any specific significance. Values given for the sugar beet " strain " vary from 0·29 mm. (Triffitt 1928) to 0·538 mm. (Liebscher 1892); for the potato " strain " one finds 0·33 mm. (Goffart 1934) to 0·52 mm. (Triffitt 1929); while the oat " strain " has been given as 0·289 mm. (Triffitt 1928) and 0·66 mm. (Liebscher 1892). However, if one compares the mean values given for the beet and oat " strains " (the potato " strain " can be ignored since it is easily identified by its rounded cysts), the two " strains " do appear to differ from one another in spite of the great variation in the figures given by the several authors. For the beet " strain " we find 0·447 mm. (Goffart 1930), 0·457 mm. (Triffitt 1929) and 0·470 mm. (O. Schmidt 1930), while in cysts from oats the larval lengths are said to be 0·470 mm.

(Triffitt 1929), 0·527 mm. (Liebscher 1892), 0·560 mm. (Goffart 1930) and 0·575 mm (O. Schmidt 1930). The general impression is that larvae of the beet " strain " of *Heterodera* are usually noticeably shorter than those of the oat " strain," but that there may be considerable variation within both " strains."

One is led to speculate upon the causes and extent of these variations. It might be that the variety of the host plant, the climate during the development of the cyst and the general vigour of the host cause variations in the length of the larvae in the cysts developed on it. If one studies the literature carefully with the object of discovering whether any of these details are mentioned one usually seeks in vain. Neither does one find details of the condition of the cysts (i.e. whether they were soaked or dry) or the number of cysts from which the larvae which were measured were obtained, while in some cases there is no mention of the number of larvae measured or the method of measuring them. It is thus impossible to compare the published larval measurements critically with one another, since they were not made under similar conditions, and without such comparison it is impossible to decide whether this character can be used for diagnostic purposes. The present authors, therefore, embarked, quite lightheartedly, on a programme of larval measuring designed to determine parameters for the lengths of larvae of the different species of *Heterodera,* which would be sufficiently accurate for comparison with similar parameters obtained by other workers for other populations of the eelworm. It was hoped, and results so far obtained justify the hope that, knowing the constants descriptive of the distribution of the larval length in each species, it would be possible to identify single cysts from the length of the contained larvae. In addition it was hoped that, when measuring soil infectivity by the number of larvae present (Fenwick 1942), it would be possible to obtain by means of larval measurements a differential estimate of the relative proportions of each species of *Heterodera* in a soil containing more than one species. The undertaking was found to be somewhat more weighty than had been anticipated, involving a matter of at least 15,000 measurements of larvae from a single species, *H. rostochiensis,* before a satisfactory technique was elaborated. But the authors are satisfied that without this preliminary work no sound basis existed upon which to start an investigation of the ultimate object, that is, the possibility of identifying single cysts or populations of larvae obtained from soil samples.

PROCEDURE.

The operations necessary in determining the parameters for the length of larvae of a species of *Heterodera* comprise three phases. The first phase consists of the choice of cysts from which larvae are to be measured and the determination of the number of larvae to be measured from each cyst. If one has set out to determine the parameters for the larval length of a given species of *Heterodera* it is obviously important to be absolutely certain of the origin of the larvae. This implies that the cysts from which they are obtained should be picked off individually from the roots of the host plants. These cysts must be mature and must contain fully developed, healthy larvae. They must also be kept damp, or soaked before use, as it has been observed that a certain amount of shrinking of the larvae occurs when cysts have been stored dry for some time. When the cysts have been soaked in water for a few days this condition usually disappears. It is next necessary to decide how many larvae should be measured from each cyst, how many cysts should be taken from each plant, and from how many plants cysts should be taken. These questions, and also the question of the variety of the host and the conditions under which it grew may or may not affect the parameters for the larval length of the parasite, but they require investigation before it can be said that they are of no importance. They fall under the head of "Sampling" and are fully dealt with later in this paper.

Having assembled the required cysts it is necessary to obtain from them the desired number of larvae in a sound and undamaged condition and to prepare them for measurement by the chosen method.

In the third phase the larvae must be mounted and measured under conditions which can be exactly repeated at any time and by other workers, and which are not too tedious to permit of large numbers of measurements being made in a short time.

These three phases, though performed in practice in the order in which they are given above, are ultimately dependent on the last operation, i.e. the actual measurement of individual larvae. They are, therefore, dealt with in reverse order in the following sections, under the headings: I. Technique of Measuring; II. Preparation of the Larvae; III. Sampling.

I. TECHNIQUE OF MEASURING.

Method of Measuring.—After trying several methods of measuring larvae, it was found that the most convenient was by means of a

calibrated eyepiece standardised against a graduated slide. The optical equipment consisted of a x6 eyepiece and a 2/3 in. objective. The standardisation of this equipment was carried out by three operators each using three calibrated slides. Five readings were made by each operator for each slide, over different regions of the scale. Each of the 45 readings so obtained was expressed as the number of divisions of the slide equivalent to one division on the eyepiece. The results

TABLE 1.
Calibration of micrometer eyepiece.

Operator	Divisions of slide equivalent to one division on eyepiece								
	A			B			C		
Slide	1	2	3	1	2	3	1	2	3
	1·6833	1·6842	1·6727	1·6842	1·6842	1·6500	1·6667	1·6780	1·6667
	1·6800	1·6842	1·6667	1·6800	1·6852	1·6667	1·6818	1·6842	1·6667
	1·6735	1·6842	1·6724	1·6800	1·6667	1·6667	1·6667	1·6552	1·6667
	1·6800	1·6842	1·6800	1·6667	1·6250	1·6774	1·6667	1·6786	1·6875
	1·6800	1·6842	1·6539	1·6800	1·6852	1·6667	1·6800	1·6667	1·6667

Analysis of Variance.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	·05492313	36	·00152 (V_1)	—
Blocks	·00409828	8	·00051 (V_2)	2·98 (V_1/V_2)
Slides	·00067287	2	·000336 (V_3)	4·53 (V_1/V_3)
Operators ...	·00049152	2	·000245 (V_4)	6·62 (V_1/V_4)
Interaction S×O	·00293389	4	·000733 (V_5)	2·08 (V_1/V_5)
Total	·05902151	44	·001341 (V_6)	—

obtained, and an analysis of variance, are set out in Table 1. Study of the analysis shows that none of the variances appropriate to slides, operators or the interaction of operator and slide is significantly greater than the error variance, and all the data may accordingly be treated as one series with a variance of ·00134139 divn.^{2}, and a mean of 1·6732 divn.. Since each division of the slide = 10μ, each division of the eyepiece must equal 16·732μ, this figure being subject to a standard error of ·005μ, i.e. ·0299% of itself. At a later stage in the investigation a number 4 objective was lent to the authors and

this proved vastly superior to the 2/3 in. lens when used with the x6 eyepiece. On calibration by the method outlined above it was found that one division of the eyepiece then corresponded to 9·2638μ, with a standard error of ·004858μ, i.e. ·0417% of itself.

Whichever objective was used it soon became apparent that the maximum precision obtainable was to the nearest whole scale division. Attempts to measure to a fraction of a division failed because the person measuring was involuntarily biased in favour of the grosser fractions and wholes at the expense of the finer fractions, i.e. in favour of halves and wholes rather than quarters. Considerations of space and paper economy prohibit the inclusion in this paper of the many and varied results obtained when fractions were taken into account; suffice it to state that the predominance of halves and wholes at the expense of quarters in any collection of data was sufficiently marked to upset the normality of otherwise perfectly normal distributions to a significant degree. It was frequently found that the number of values ending in ·0 and ·5 in a distribution was double the number ending in ·25 and ·75. Similarly, but to a lesser extent, when measurements were made to the nearest half division, values ending in ·0 predominated over those ending in ·5. A rough estimate of the chances of obtaining distributions comparable in form and consistency with those obtained by the authors indicated a probability of one in several hundred—a chance so slender as to condemn the results out of hand. The difficulty of measuring to any fraction of a division is apparent when one considers what the process implies. Suppose it is decided to measure to the nearest quarter of a division; it is obviously necessary that the person measuring should be capable of estimating the $\frac{1}{8}$ marks, since any single value must be considered as lying between two alternate $\frac{1}{8}$ marks, i.e. $\frac{1}{4}$ will comprise all values between $\frac{1}{8}$ and $\frac{3}{8}$, $\frac{1}{2}$ includes those from $\frac{3}{8}$ to $\frac{5}{8}$, and $\frac{3}{4}$ will be values between $\frac{5}{8}$ and $\frac{7}{8}$. This is the inevitable result if the figure quoted for a group length be its mean. The position may be somewhat improved if one specifies instead of the group mean its higher or lower limit. In this case one half of the group interval must be added to or subtracted from the general mean obtained by taking group limits: if it is desired to measure to half a division, measurements lying between ·0 and ·5 will be recorded as ·5, and values intermediate between ·5 and ·0 will be recorded as ·0, say. The true mean will then be obtained by summing the recorded data, dividing by the number in the sample, and subtracting 0·25.

This was the technique finally adopted for the present investigation. Measurements were made to a whole division on the micrometer eyepiece scale, and all lengths intermediate between two divisions were given the value of the next higher division; 0·5 was substracted from the mean of all values to obtain the true grand mean of a sample. The effect of this technique on the parameters appropriate to any sample is worth considering; if x_1, x_2, x_3,———x_n represent n individual values, then $(x_1-0{\cdot}5)$, $(x_2-0{\cdot}5)$ etc. will represent group means, and the true general mean will be $\left(\frac{Sx}{n}-0{\cdot}5\right)$, where Sx indicates the summation over all values of x. When computing higher moments however, it is unnecessary to apply any corrections, since the deviation of group values from $\left(\frac{Sx}{n}\right)$ is the same as the deviation of the true group means, the latter two measurements being each 0·5 divisions less than the former. In fact, for all statistical computations which do not necessitate a direct and *absolute* estimate of the mean, no error will result from assuming the group mean to be the group limit, and the term $\left(\frac{Sx}{n}\right)$ to be the general mean. The sole result of such a procedure will be to shift the position of the frequency curve 0·5 divisions in a positive direction without in any way altering its shape.

There is one point worthy of attention regarding the statistical treatment of data when the measurements are made to any given standard of accuracy (to the nearest whole division in this case); it has the effect of grouping the data, since of necessity, it has to be assumed that all the frequencies for one group are concentrated at its mean. When such data are analysed statistically, corrections have to be made to allow for this loss of information by grouping, the most commonly used being Sheppard's corrections, which are applied to moments of even number. In the case of the second moment the correction consists in subtracting one twelfth of the group interval squared from the moment as computed from the grouped data. This correction is sufficient provided that the group interval is small compared with the standard deviation. In this case, however, as will be seen on studying the data which follow, the group interval is not very much smaller than the standard deviation, and the question arises, are Sheppard's corrections sufficient? This can be answered by comparing the errors incurred by neglecting further corrections with the standard error of the particular parameter involved. Let k be the error incurred,

expressed as a fraction of the standard error of the parameter, for cases where the group interval is of the same magnitude as the standard deviation. Then $\frac{\sigma}{\sqrt{n}} k = \frac{\sigma}{\pi} e^{-2\pi^2}$ where n = number of individual readings and σ = standard deviation; whence k = ·000,000,00814, while for the second moment $\frac{2\sigma^2}{\sqrt{n}} = 4\sigma^2 e^{-2\pi^2}$ and k = ·000,000,07123, for values of n = 100. Thus, for the first and second moments the errors are seen to be of a very low order compared with the standard errors of these parameters, and the loss in consistency is so small that further corrections can be neglected. For other values of n, k can be computed. On study of the above equations it is obvious that k is in both cases proportional to $\sqrt{n}$ for any given set of parameters, and obviously an extremely large number of measurements would have to be made for k to reach an appreciable value.

Mounting of the larvae for measurement.—Whilst it is obvious that the most reliable method of mounting larvae for microscopical examination is on a slide in a drop of water under a coverglass, this method was unsuitable for the present purpose, since it is well nigh impossible in this way to arrange large numbers of larvae in compact groups for quick measurement. On the other hand, inconsistent results were given when the larvae were measured in drops of water without a coverglass. Different values were often obtained for the same larva measured in different drops, the length apparently being partly governed by the size of the drop. The possible causes of discrepancies lie in the depth of the drop and the curvature of its surface: it is obvious that a highly curved drop of water will act as a convex lens and magnify the larva, the degree of magnification being dependent primarily on the curvature of the drop and secondarily on its depth. In general it may be said that the more convex and the deeper the drop, the greater will be the error incurred. If the drop is spread out so that its surface is flat, it is obvious that there can be no magnification, since the focal length of the drop would be infinity, though a larva viewed through the edge of the drop, where the water surface is curved, would appear distorted. Over the regions of the drop where the surface is flat its depth would have no effect.

In order to investigate the effect of the depth and curvature of the drop of water in which a larva is measured, ten larvae were mounted in three different ways and measured. The three methods were:

—(*a*) " deep drop "—a small drop of water with a very convex surface; (*b*) " spread drop "—a very extensive drop with a practically horizontal surface; (*c*) " coverslip "—a drop of water covered with a covership in the usual way. Each larva, on a numbered slide, was measured in each type of drop, the slides were shuffled, the drops altered, and the larvae again measured. The process was repeated until each larva had been measured five times in each type of drop. The results of this experiment, together with analyses of variance, are set out in Table 2.

TABLE 2.

Effect of method of mounting on the apparent length of larvae.*

		Apparent length of larvae (micrometer divisions)										Treatment totals
Larva no.		1	2	3	4	5	6	7	8	9	10	
Deep drop	1	33	34	37	37	36	37	33	31	32	33	
	2	31	35	34	36	34	34	32	31	31	32	
	3	34	36	37	37	34	35	35	36	38	35	
	4	31	31	33	32	33	34	33	34	33	33	
	5	32	31	33	32	33	34	33	34	33	33	
Block Total		161	167	175	175	171	173	165	164	166	165	1682
Spread drop	1	29	29	30	30	30	29	29	30	31	30	
	2	28	28	30	30	30	29	29	29	31	30	
	3	29	29	30	30	30	29	29	29	31	30	
	4	28	29	30	30	30	29	29	29	31	30	
	5	29	29	30	30	30	30	29	29	31	30	
Block Total		143	144	150	150	150	146	145	146	155	150	1479
Coverslip	1	28	29	30	30	30	29	29	29	31	30	
	2	28	29	30	30	30	29	29	29	31	30	
	3	28	29	30	30	30	29	29	29	31	30	
	4	28	28	30	30	30	29	29	29	31	30	
	5	29	29	30	30	30	29	29	29	31	30	
Block Total		141	144	150	150	150	145	145	145	155	150	1476
Larva Total		445	455	475	475	471	464	455	456	476	465	4637

General Analysis of Variance.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Treatment ...	557·6933	2	278·847 (V_1)	164·9 (V_1/V_3)
Larvae	68·1403	9	7·571 (V_2)	—
Interaction L×T	30·4397	18	1·6911 (V_3)	14·876 (V_3/V_4)
Error	13·6	120	0·1133 (V_4)	—
Total	669·8733	149	—	—

Analysis of Variance on spread drop and coverslip data.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Treatment ...	0·09	1	0·09 (V_1)	1·2 (V_1/V_4)
Larvae	54·25	9	6·03 (V_2)	—
Interaction L×T	0·41	9	0·045 (V_3)	—
Error	6·0	80	0·075 (V_4)	—
Total	60·75	99	—	—

* Larval lengths in all tables are in terms of divisions of the micrometer eyepiece.

From the general analysis of variance it will be seen that the treatment variance is significantly greater than either the interaction or error variance. Since the treatment totals are based on 50 values each, the critical difference between them will be 6·735, and it will be seen that " deep drop " measurements are significantly greater than either of the other two sets, which latter do not differ significantly from one another. Study of the data, however, shows that the error variance of " deep drop " measurements is greater than that of the others and it was felt that the mean error variance in the general analysis of variance might not be valid for comparing totals for " spread drop " and " coverslip " techniques, hence a further analysis of variance was performed on the latter. It will be seen that, as a result, the error variance has been reduced from 0·1133 to 0·075, but even so no significant difference can be detected between the " spread drop " and the " coverslip " techniques.

It was accordingly decided that the " spread drop " technique be adopted, and to this end, on a number of slides four walls of wax forming a rectangle about one inch square were built. The larvae were

placed in the centre of the cavity thus formed and water was added to fill it and give a flat surface; if too much water was added producing a convex surface, excess was withdrawn until the surface became flat. The larvae were arranged in a compact group near the centre of the cavity, no larvae near the edges being measured.

II. Preparation of the Larvae.

The larvae may be obtained from a cyst either by physical or by chemical means. In the former case the cyst may be dissected with needles, or squashed between two slides and the eggs so liberated may be burst open under a coverslip by gentle pressure so as to free the larvae without damage. A certain number of the larvae may be squashed in this process, but with practice this number can be reduced to a very small proportion of the whole. This method is referred to as the " squash technique " for recovering larvae, and has the advantage of being fairly rapid and of requiring very simple apparatus.

Stimulation by the root excretions of the host plants will cause hatching of the larvae of some species of *Heterodera,* but this may take several days, and it is impossible to tell what proportion of the larvae in each cyst has hatched, or whether the larvae have been derived from all or only some of the cysts in a batch. In addition, the larvae so obtained have hatched over a period of several days, and the method cannot be used for all *Heterodera* species, since some are apparently not stimulated by excretions from the roots of their hosts. This method of obtaining larvae is therefore useless when exact knowledge of their history and origin is required. Measurements of larvae hatched by means of root excretion gave such erratic results as to be worthless for the present work.

A much more satisfactory chemical means of obtaining *Heterodera* larvae is by the use of calcium hypochlorite solution. If the soaked cysts are crushed in a drop of water the eggs are easily separated and transferred to a watchglass, where they are concentrated into a small drop of water and treated with a very dilute calcium hypochlorite solution. In a few minutes (depending on the strength of the solution) the larvae burst out of the egg shells and the hypochlorite is then replaced by water to prevent chemical damage to the larvae. By this method practically all the larvae in a batch of cysts can be recovered in apparently good condition.

The larvae obtained by either the " squash " or the hypochlorite technique should be living, provided that the cysts were healthy and

mature, and they will probably be moving, or at least to some extent bent. If they are to be measured against a straight scale, which is the most convenient method when large numbers are to be measured, and is the one adopted by the authors, it is necessary to make them straighten. There are several methods of killing or fixing larvae in a relaxed or straight position. Schmidt (1930) killed and stained in a solution of iodine in potassium iodide. Alternatively, if larvae in a drop of water are exposed to an atmosphere containing a strong concentration of chloroform vapour for half to one hour they become quiescent and straight. However, the most usual method of fixing nematodes is by gentle heat, and as this is a very satisfactory way of preparing *Heterodera* larvae for measurement, it was adopted for the present work.

When it had been decided to determine the larval length for the several species of *Heterodera,* larvae were recovered from the cysts by both the squashing and the hypochlorite techniques, and were fixed by means of boiling water which was poured into the vessel containing them. Measurements of a number of larvae from batches thus obtained were very carefully made according to the measuring technique described above, with every expectation that the results would be consistent. However, this optimism proved to be ill-founded, as it soon became evident that the variance between batches of larvae treated apparently identically was greater than the error variance. It was therefore necessary to investigate fully the details of the two techniques.

Effect of the technique of recovering and fixing larvae on their length.

The factors in the preparation of larvae for measurement which might affect their length may be classified as follows :—

(*a*) Conditions common to the two techniques.

(*b*) Conditions peculiar to the hypochlorite technique.

(*c*) Conditions peculiar to the " squash " technique.

(*d*) Time of measurement in relation to " hatching " and fixing of the larvae.

(*a*) *Conditions common to both techniques—temperature of fixation.*

In order to investigate the effect of the temperature of fixation on larval length, 400-500 cysts were taken and the larvae recovered from them by the hypochlorite method. All the larvae for the following experiment were drawn from this one batch. In order to control accurately the temperature of fixation, a water bath was maintained

at the required temperature. About 100 larvae, in the least possible quantity of water, were placed in a small specimen tube which was then plunged into the bath, and water from the bath was added to

TABLE 3.

The effect of temperature of fixation on the length of larvae.*

Temperature group	A (100°C.)			B (90°C.)			C (80°C.)			D (70°C.)		
L	1	2	3	1	2	3	1	2	3	1	2	3
24	2	7	—	—	—	—	—	—	—	—	—	—
25	2	3	3	—	1	2	—	—	—	—	—	—
26	4	5	3	—	3	1	—	—	—	—	—	—
27	6	5	4	—	4	5	—	—	2	2	—	—
28	3	—	5	1	5	8	2	1	—	—	4	2
29	3	—	2	6	4	3	6	2	4	6	6	4
30	—	—	3	8	3	1	7	8	6	7	5	7
31	—	—	—	2	—	—	4	5	5	2	4	5
32	—	—	—	3	—	—	1	3	2	3	1	1
33	—	—	—	—	—	—	—	1	1	—	—	—
Total	535	508	549	600	557	552	596	610	602	596	592	602
Temp. total	1592			1709			1808			1790		

General Analysis of Variance.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	404·65	228	1·7748 (V_1)	—
Blocks	120·533	8	15·06 (V_2)	8·48 (V_2/V_1)
Sets	775·98	3	258·66 (V_3)	145·7 (V_3/V_1)
Total	1301·1625	239	—	—

* In this table and in other similar tables which follow, the values in the column marked "L" represent the higher limits of group lengths in terms of the divisions of the micrometer eyepiece. Figures in horizontal rows corresponding to each value of L give numbers of larvae which fall into the individual group. The totals at the foot of each column give the sum of the lengths of all larvae in the column.

Analysis of Variance on A and B.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	218·85	114	1·91 (V_1)	—
Blocks	113·57	4	28·39 (V_2)	14·87 (V_2/V_1)
Sets	115·573	1	113·57 (V_3)	59·6 (V_3/V_1)
Total	445·998	119	—	—

Analysis of Variance on C and D.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	185·8	114	1·623 (V_1)	—
Blocks	7·53	4	1·875 (V_2)	1·12 (V_2/V_1)
Sets	2·637	1	2·637 (V_3)	1·644 (V_3/V_1)
Total	196·967	119	—	—

the larvae by means of a pipette which had been immersed in the bath for several minutes before use. The larvae were held at the required temperature for 30 seconds, allowed to cool, and then transferred to a measuring slide and measured. Three samples of larvae were submitted to each temperature, 20 larvae from each sample being measured. Experiments were conducted at 100° C. (A), 90° C. (B), 80° C. (C) and 70° C. (D). The results are set out in Table 3 with analyses of variance. Each batch of 20 larvae is referred to in the analysis as a block, whilst each set of three blocks is called a set. It will be seen from the general analysis that the variance of blocks within sets is significantly greater than error, while the variance of sets is greater than that of blocks. In other words, the variances due to error, blocks and sets respectively are in ascending order of magnitude, showing that, generally speaking, the results are inconsistent from block to block, while the variations due to temperature differences are greater even than block variations. This is conclusive evidence that the temperature of fixation affects the length of the larva. Examination of the data shows that larvae fixed at temperatures above 80° C. are shorter than those fixed at lower temperatures.

If the data are analysed in two parts, one analysis being performed on the figures for fixation at 90° C. and 100° C., the other on those

for 70° C. and 80° C., it is obvious that, for the higher temperatures the variances are still in significantly ascending order of magnitude, the " between-block " variance being significantly greater than the error variance, and the " between-set " variance greater than either, but for the lower temperatures none of the variances is significantly greater than the error, and all the results for 70° C. and 80° C. therefore fall into one single consistent series.

The results of the investigation into the effects of temperature of fixation on larval length may accordingly be summarised as follows :— temperatures above 80° C. yield inconsistent " between-block " results, while the results obtained with lower temperatures are consistent. Larvae fixed at temperatures above 80° C. are significantly shorter than those fixed below this temperature. It is accordingly recommended that when larvae are to be fixed for measuring, they should be fixed in water between 70° C. and 80° C. In practice it was found convenient to place the larvae in the smallest possible quantity of water in a solid watchglass and to add to this a few ccs. of water at approximately 75° C. Nearly all the larvae in a batch treated in this way straighten out immediately, apparently being killed, and are in excellent condition for measuring.

(*b*) *Experiments on the hypochlorite technique.*

The hypochlorite technique in its original form was a very convenient means of obtaining larvae, but it was felt that the conditions were not sufficiently well defined. Controlled experiments were therefore carried out in which liberated eggs were subjected to varying strengths of hypochlorite for varying times. In these experiments the precautions regarding the handling of hypochlorite solutions described by Fenwick in a previous paper (1942) were stringently observed. Considerations of space prohibit the inclusion of the many results obtained, some consistent, others not, but two satisfactory methods are described here. In each case blocks of 50 cysts were used which were squeezed between two slides until all the eggs were freed. Provided that care was exercised in the squeezing, very few larvae were set free from the eggs, and of these few the number crushed was negligible.

The two methods to be described differ from one another chiefly in the strength of the hypochlorite used. In the first method the egg suspension was made up to 2 ccs. with distilled water, and to this

was added 0.2 ccs. of 5% B.D.H. bleaching powder solution containing about 1% available chlorine. The eggs were treated with this solution for ten minutes, then fixed with hot distilled water at 75–80° C. To allow for possible swelling of the larvae during the time they were kept in distilled water after liberation from the egg shells, they were measured 15 minutes and 24 hours after "hatching," being kept for the intervening period in an incubator at 28° C. The results of this experiment (method 1) and of experiments on the second method (method 2) are set out in Table 4.

The second method differed from the first in that the eggs, in the smallest possible quantity of water, were transferred to a few ccs. of hypochlorite solution made up by adding 7·5 ccs. of the above mentioned 5% bleaching powder solution to 50 ccs. of distilled water. The eggs were treated in this solution for ten minutes, the liberated larvae were then fixed and measured as before. In both methods very stringent precautions were taken to ensure that the larvae were well washed with distilled water to remove any free hypochlorite, the washing being accomplished in the second method by centrifugation. Centrifugation was later found to be unnecessary, it being sufficient merely to add fresh water, allow the larvae to settle, then remove the supernatent liquid, repeating the process three or four times. The difference in strength of the two solutions can best be appreciated from chemical analyses based on several determinations made at different times during the series of experiments. The solution for the first method was found to contain approximately 0·087% available chlorine, while that for the second contained approximately 0·126%.

An analysis of variance of the larval measurements obtained shows that for both techniques there is no significant difference between the lengths of different blocks of larvae which had received the same treatment. It will be observed that five blocks of larvae were measured for each time and each treatment, the five blocks constituting a "set," and 50 larvae were measured in each block. The variance between sets proved to be significantly greater than the error or the block variance. Further analysis of the set variance into its constituent parts shows it to be made up of variances due to the effects of time, method and interaction of method with time (V_4, V_5 and V_6 respectively). Of these the only variance significantly greater than error is that due to time (V_4), which is also greater than the interaction (V_6). Therefore the only factor which contributes to set variance is time.

We may therefore conclude that there is no significant difference between the techniques, no significant interaction, both techniques are capable of giving consistent results, and larvae obtained by both techniques are longer after being kept for 24 hours than they are 15 minutes after liberation from the eggs.

TABLE 4.

Test on two hypochlorite techniques combined.

L	Method 1										Method 2									
	15 min.					24 hours					15 min.					24 hours				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
25	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—
26	—	—	1	1	—	1	—	—	—	1	—	1	1	1	—	2	—	—	1	1
27	1	1	2	1	—	2	—	1	2	1	2	4	2	—	2	4	—	1	3	1
28	5	6	4	4	5	3	4	1	4	5	9	3	6	4	4	6	5	4	4	5
29	11	13	14	12	13	7	9	7	7	2	13	8	12	17	18	7	8	4	7	9
30	17	18	16	18	20	6	9	14	8	19	14	18	11	17	18	8	11	10	13	18
31	9	7	8	10	7	9	18	18	9	12	8	12	12	9	4	10	5	9	8	5
32	2	4	4	4	3	13	6	5	13	8	1	3	5	2	3	10	14	14	11	4
33	4	1	1	—	1	6	—	3	5	2	2	1	1	—	1	2	4	4	3	4
34	1	—	—	—	1	1	3	1	1	—	1	—	—	—	—	—	3	3	—	1
35	—	—	—	—	—	1	1	—	1	—	—	—	—	—	—	—	—	1	—	2
Sx	1505	1490	1487	1491	1497	1534	1530	1529	1538	1515	1483	1491	1491	1484	1481	1492	1539	1551	1511	1513

Analysis of Variance.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	2634·1	980	2·6268 (V_1)	—
Blocks	56·492	16	3·53075(V_2)	1·345 (V_2/V_1)
Sets	130·304	3	43·434 (V_3)	16·48 (V_3/V_1)
Time	122·90	1	122·90 (V_4)	46·7 (V_4/V_1) 30·5 (V_4/V_2)
Method	5·40	1	5·4 (V_5)	2·05 (V_5/V_1)
Interaction M×T	4·004	1	4·004 (V_6)	1·57 (V_6/V_1)
Total	28320·896	999	—	—

(*c*) *Experiments on the "squash" technique.*

Careful consideration of the "squash" technique led to the conclusion that the only factor which could have given rise to the inconsistency of the previous results must be mechanical damage to the larvae. It can easily be shown that crushed larvae are shorter than undamaged ones. This shortening may occur in larvae which have received such slight local damage to the cuticle that it is only visible on very careful examination. When examining damaged larvae some exudation may be observed at the point of injury; this was found to be a reliable symptom of damage even when the damage itself was not readily apparent. Very careful experiments were therefore made using the "squash" technique, particular attention being paid to the prevention of mechanical damage, and great care being taken that no larva showing any suspicion of damage was measured. The larvae were fixed as in the hypochlorite method by the addition of water at 75–80° C. As before, samples were based on larvae recovered from blocks of 50 cysts, 50 larvae being measured per block and five blocks constituting a set. Two series of measurements were made, one 30 minutes after squashing, the other 24 hours later. The results are set out in Table 5 together with an analysis of variance. It will be seen that block variance is significantly greater than error variance, if the 5% limits of significance are accepted, but only barely so. Perusal of the data will show that this is almost entirely due to one abnormally low value in the 24 hour measurements (block No. 3). If this be disregarded, a further analysis will show no significant difference between blocks. The time variance is seen to be highly significant, the

24 hour measurements being substantially higher than the 30 minute ones, a finding in accordance with the hypochlorite results.

Comparison of hypochlorite and " squash " techniques.

It was decided at this stage, in view of the more consistent results now obtainable with the two techniques, that before proceeding to investigate factors such as the influence of the time interval between hatching and measuring, etc., a detailed comparison should be made of the hypochlorite and " squash " techniques. To this end a detailed analysis of variance was performed on the data in Tables 4 and 5, the

TABLE 5.

Test on the " Squash " Technique.

	30 minutes					24 hours				
L	1	2	3	4	5	1	2	3	4	5
25	—	—	—	—	—	—	—	—	—	1
26	—	—	—	—	—	—	—	—	—	—
27	—	1	—	—	1	—	—	—	1	—
28	2	—	3	1	2	1	—	—	1	1
29	10	11	11	3	8	1	1	6	2	4
30	20	12	15	17	11	2	8	12	3	4
31	12	14	14	20	18	16	17	13	10	5
32	5	7	7	6	9	19	12	11	14	13
33	1	5	—	3	1	8	8	6	16	10
34	—	—	—	—	—	3	3	2	1	9
35	—	—	—	—	—	—	1	—	2	—
	1511	1529	1511	1536	1524	1587	1581	1555	1593	1589

Analysis of Variance.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	854·4	490	1·758 (V_1)	—
Blocks	28·6	8	3·527 (V_2)	2·012 (V_2/V_1)
Sets	72·872	1	72·872 (V_3)	41·4 (V_3/V_1)
Total	955·872	499	—	—

data being grouped together and treated as one experiment. For the purposes of this comparison the fact that the first measurements of the larvae liberated by squashing were made 30 minutes after liberation, while those liberated by hypochlorite were first measured 15 minutes after being freed, is ignored, since this difference is relatively trifling. The analysis of the combined results is set out in Table 6. It will be seen that all variances above block variance are significantly greater than that

TABLE 6.

Analysis of Variance on hypochlorite and "squash" techniques together.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	3222·5	1470	2·19 (V_1)	—
Blocks	4·708	24	3·505 (V_2)	1·60 (V_2/V_1)
Sets	511·309	5	111·87 (V_3)	51·1 (V_3/V_1) 31·9 (V_3/V_2)
Time	278·2107	1	278·21 (V_4)	127·0 (V_4/V_1) 5·25 (V_4/V_6)
Method	123·989	2	61·99 (V_5)	28·3 (V_5/V_1) 1·16 (V_5/V_6)
Interaction T×M	159·1093	2	79·555 (V_6)	31·3 (V_6/V_1)
Total	3868·5174	1499	—	—

ascribable either to error or to blocks, but that block variance (V_2) itself is not greater than error variance (V_1). The subdivision of the set variance into time, method and interaction variances (V_4, V_5 and V_6 respectively) discloses the fact that the interaction variance (V_6) is significantly greater than either error or block variance (V_1 or V_2). The variances due to time and method (V_4 and V_5) are neither of them significantly greater than the interaction (V_6). It was therefore necessary to investigate the action of each of these three factors in conjunction with one another. It was found that for both periods of time and also in general the "squash" technique gives longer larvae than do the hypochlorite techniques, but that there is no significant difference between the two latter at either time nor in general. The larvae obtained by all three techniques are longer 24 hours than they are 15 minutes after "hatch-

ing," but the increase in length is greater with the " squash " than with the hypochlorite techniques. All three methods give consistent " blocks-within-sets " results.

The foregoing results, however, while interesting, cannot be considered conclusive, especially as each technique involved only one operator, one author carrying out the two hypochlorite methods whilst the other used the " squash " method, and previous experiments (the results of which are not published for considerations of space) showed that it was possible under certain circumstances for the effect of operators to be very well marked. In fact, in one experiment when the techniques were tried out in an incompletely developed form, one author had higher results than the other for one technique, but lower for the other technique. It was accordingly decided that, with the co-operation of other members of the staff, it would be useful to carry out an experiment including six operators, each of whom should perform both techniques on five blocks of cysts. In this way it would be possible to compare the " squash " with one of the hypochlorite techniques thoroughly and exhaustively. For this purpose the two techniques were standardised and the larvae measured 30 minutes after liberation from the eggs. It was purposely arranged that not all the operators should be experienced in this type of work. In fact, two of them had no experience whatever of eelworm work, while a third was completely inexperienced in measuring. Considerations of space do not permit the inclusion of all the results in detail, but the block totals for each operator together with the appropriate sums of squares taken from zero are given in Table 7, together with analyses of variance. In the first general analysis it will be seen that all variances are significantly greater than error—a most unsatisfactory state of affairs. Closer and more detailed analysis disclosed the fact, however, that the high mean variance between blocks was due to inconsistencies on the part of operator A, and a second analysis is given omitting operator A's results. It will be seen that now there is no significant difference between blocks, but that sets do vary significantly. The interaction variance is significantly greater than error, but neither the variance due to operators nor to methods is significantly greater than interaction, although both are greater than error. It is therefore necessary to investigate the effect of these latter in relation to one another, and the appropriate totals, together with critical differences, are set out below the analysis. It will be seen that in only one case is there any significant difference between operators—D has obtained lower results for both techniques generally speaking than

the other operators. There are, also, two significant interactions; in the first place B has obtained rather high results for the hypochlorite technique, and secondly F has low results for the " squash " technique. Summarising the results of this " operator " experiment, it may be concluded that there is no real difference between the two techniques.

TABLE 7.

Test of Techniques in the hands of six operators.

Operator		"Squash"					Hypochlorite				
		1	2	3	4	5	1	2	3	4	5
A	Sx	1,440	1,471	1,455	1,501	1,483	1,477	1,435	1,451	1,479	1,481
	Sx^2	41,540	43,351	42,401	45,165	44,061	43,721	41,253	42,185	43,815	43,959
B	Sx	1,463	1,454	1,450	1,474	1,460	1,498	1,517	1,501	1,500	1,508
	Sx^2	42,889	42,354	42,132	43,546	42,690	44,928	46,139	45,117	45,076	45,542
C	Sx	1,492	1,491	1,479	1,484	1,494	1,479	1,481	1,485	1,497	1,480
	Sx^2	44,570	44,531	43,815	44,128	44,686	43,847	43,951	44,193	44,915	43,886
D	Sx	1,443	1,463	1,451	1,456	1,447	1,452	1,457	1,470	1,462	1,464
	Sx^2	41,705	42,865	42,187	42,444	41,965	42,260	42,535	43,318	42,806	42,908
E	Sx	1,479	1,485	1,478	1,482	1,468	1,477	1,471	1,479	1,507	1,470
	Sx^2	43,807	44,169	43,758	44,020	43,194	43,747	43,363	43,859	45,529	43,294
F	Sx	1,455	1,435	1,444	1,452	1,437	1,489	1,492	1,473	1,463	1,469
	Sx^2	42,413	41,261	41,778	42,236	41,391	44,417	44,606	43,465	42,857	43,235

Analysis of Variance.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	4590·72	2940	1·5615 (V_1)	—
Blocks	147·600	48	3·075 (V_2)	1·96 (V_2/V_1)
Sets	310·047	11	28·186 (V_3)	9·2 (V_3/V_1)
Method	52·802	1	52·802 (V_4)	34·8 (V_4/V_1) 2·46 (V_4/V_6)
Operators ...	151·006	5	30·201 (V_5)	19·4 (V_5/V_1) 1·49 (V_5/V_6)
Interaction O×M	106·221	5	21·24 (V_6)	13·6 (V_5/V_1)
Total	5048·361	2999	—	—

Analysis of Variance omitting A.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	3813·98	2450	1·5567 (V_1)	—
Blocks	68·656	40	1·7164 (V_2)	1·11 (V_2/V_1)
Sets	302·905	9	33·656 (V_3)	21·65 (V_3/V_1)
Method	72·25	1	72·25 (V_4)	46·4 (V_4/V_1) 3·4 (V_4/V_6)
Operator... ...	145·323	4	36·33 (V_5)	23·3 (V_5/V_1) 1·705 (V_5/V_6)
Interaction O×M	85·332	4	21·333 (V_6)	13·7 (V_6/V_1)
Total	4185·541	2499	—	—

Set totals with critical differences.

Treatment	B	C	D	E	F
Squash	7301	7440	7260	7392	7223
Hypochlorite	7524	7422	7305	7404	7386

Critical differences: between cells = 55·8.
between operators = 79·0.
between methods = 128·8.

The fact that the hypochlorite technique gives apparently higher results than does the " squash " is entirely due to the interactions shown by B and F.

So far no measure has been obtained of the " between-operator " consistency of the two techniques separately; all that has been derived is a value for the mean variance between the techniques. Table 8, giving analyses of variance on the two techniques separately, has been compiled in order to throw some light on this point. If error, block and operator variances respectively be designated V_1, V_2 and V_3, then since in neither case is V_2 greater than V_1, both may be taken as being different estimates of the same parameter, and they may be lumped together to obtain a more consistent estimate of this; this may be done by addition of the sums of squares, and division of this figure by the sum of the number of degrees of freedom appropriate to each.

TABLE 8.

Analyses of Variance on the " squash " and hypochlorite techniques separately.

(A) *Hypochlorite technique.*

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	2021·8	1225	1·650 (V_1)	—
Blocks	44·232	20	2·216 (V_2)	1·34 (V_2/V_1)
Operators ...	99·043	4	24·7607 (V_3)	14·97 (V_3/V_1)
Total	2164·455	1249	—	—

(B) " *Squash* " *technique.*

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	1792·8	1225	1·464 (V_1)	—
Blocks	24·43	20	1·2215 (V_2)	1·195 (V_2/V_1)
Operators ...	131·605	4	32·90125 (V_3)	21·28 (V_3/V_1)
Total	1948·835	1249	—	—

A value of 1·6589 based on 1245 degrees of freedom is thus obtained for the " within-operator " variance for the hypochlorite technique. Now, V_3 is an estimate, based on four degrees of freedom, of 250 times the variance of operator means, and is made up of two parts, (*a*) the variance of means when each is derived from an infinitely large number of measurements ($=\sigma^2$, say), and (*b*) the standard error applicable to operators' means when each is derived from 250 measurements. Therefore $V_3/250$ is the sum of (*a*) and (*b*), and $=\sigma^2+(\text{S.E.})^2$.

Now the (standard error)^2 of the operator means for the hypochlorite technique $= 1{\cdot}6589/250$, therefore $V_3/250 = \sigma^2 + 1{\cdot}6589/250$, whence $\sigma^2 = {\cdot}092443$. This is an estimate of the variance of operators' means when each is based on an infinite number of values, and being itself based on four degrees of freedom has a standard error of ·004275. By a similar method of computation the corresponding value for the " squash " technique is found to be 1·291, with a standard error of ·00799. The arithmetical difference between the two parameters is thus ·034, with a standard error of 0·1109; the difference cannot therefore be significant, i.e. neither technique gives more consistent " between-operator " results than the other.

The discrepancies in the results obtained by some of the operators bring out clearly the difficulties and dangers to be encountered when making large numbers of measurements of larvae of *Heterodera*, or, for that matter, of any nematodes of similar size. There are two main pitfalls for the inexperienced worker. The first is due to the difficulty in determining the exact termination of the rather finely pointed tail. When 50 measurements have to be made as expeditiously as possible by one who is perhaps unpractised in the manipulation of a microscope slide so as to bring the object on it into line with an eyepiece scale, it is easy, until one's eye becomes accustomed to picking out the end of the tail, to underestimate its length. In the present experiment the magnification employed (one eyepiece division $= 16{\cdot}73\mu$) tended to aggravate this difficulty, but in subsequent work this was to a great extent overcome by the use of a number 4 objective in place of the 2/3 in. one, making one eyepiece division $= 9{\cdot}26\mu$. It seems very probable that operator D, who obtained low readings with both techniques, and who had done no measurements of this kind before, fell into this particular trap. After a little practice in measuring and an opportunity of examining *Heterodera* larvae at a higher magnification, she made fresh measurements exactly as in the original experiment and obtained results agreeing with those obtained by the other operators. The second difficulty encountered lies in the choice of larvae to be measured. In the procedure adopted a large number of larvae is placed on the measuring slide and 50 of these have to be measured. Ideally one should measure the first 50 which present themselves, avoiding obviously damaged ones, but in practice it is advisable also to avoid those which are at all bent. Although bent larvae may show no signs of having been burst, and in many cases may be undamaged, if they are pressed with a fine bristle they are frequently found to have lost the normal turgidity of sound larvae and

hence have probably been damaged and are consequently shrunk. After some experience of *Heterodera* larvae one can often pick out these slightly damaged ones, partly by the fact that they are not perfectly straight, and partly by the absence of a clear definition of the internal organs. A staining method, described below, has now been developed to help in the avoidance of these unsound larvae. In view of the fact that this kind of damage to larvae is more likely to occur in the " squash " than the hypochlorite technique, and that if such larvae were measured they would give low results, and since operator F, who was unfamiliar with *Heterodera* larvae, obtained low results for the " squash " technique only, it was considered likely that this might have been due to his having measured slightly bent, unsound larvae. It was in fact found that he had been conscientiously measuring the first 50 apparently undamaged larvae encountered, irrespective of whether they were slightly bent or not. He was therefore good enough to repeat his measurements as before, but with the added refinement of the staining technique described below to assist in avoiding damaged larvae. His results then corresponded with those of the other operators, as shown below.

Of the other anomalous results, the rather high figures given by operator B in using the hypochlorite technique can probably be explained as being due to slight overestimation of the length of the larvae when the operator was making mass measurements for the first time. This operator, who has very great experience of microscopical examination of nematodes, found that the magnification stipulated was insufficient for his comfort, and suggested the use of a higher power objective. It was unfortunately considered inexpedient to adopt his suggestion at this stage of the experiment, though it has been adopted in subsequent work. When B did the " squash " technique measurements, probably having become accustomed to the magnification in use, his results agreed with those generally obtained. It is almost certain that if he were to repeat his measurements or were to make them at the higher magnification now used, his results would now conform with the others.

Only one operator, A, produced results which were inconsistent between blocks of larvae: no explanation can be offered for this, as the conditions of the experiment were most carefully observed. Possibly this operator would obtain consistent results with the aid of the staining technique given below, coupled with the use of the higher magnification, and with greater familiarity with *Heterodera* larvae.

The staining technique referred to above was unfortunately not discovered until the main " operator experiment " had been completed. It was found that a 1 :10,000 solution of neutral red would not stain living *Heterodera* larvae, even if they were submerged in it for several days. It was also found that if living larvae were fixed in water at 70–80° C. and then transferred to the stain they, too, remained colourless, but larvae fixed at 90° C. or over immediately took up the stain, and on being transferred to clear water could easily be distinguished from larvae fixed at lower temperatures. In addition, if larvae which have sustained mechanical damage are soaked in neutral red for 10 minutes, the stain will be found to have penetrated the ruptured cuticle. Neutral red will also stain the anterior end of larvae which have been damaged by hypochlorite solution, the mouth apparently being rendered permeable to the stain. It will thus be obvious that if a sample of larvae is treated for 10 minutes with a 1 :10,000 solution of neutral red and then transferred to clear water it will be possible to pick out any which may have been damaged by heat or by hypochlorite or have burst. Neutral red is therefore extremely useful in avoiding the measuring of larvae damaged in any of these ways. It has one other great advantage when the hypochlorite technique is being used for liberating larvae—it is decolourised by hypochlorite. It therefore serves as a very useful indicator of excess hypochlorite in a larval suspension. In view of the above properties of neutral red the following experiments were made to test its usefulness in practice.

Staining test.

Three sets of five blocks of 50 cysts were taken and the larvae recovered from them by the hypochlorite technique. The three sets were treated as follows :—the first five blocks were prepared carefully and the larvae were fixed at 70–75° C. (A), the second five blocks were prepared equally carefully but the larvae were fixed at 100° C. for three minutes (B), the third five blocks were treated with 5% hypochlorite for a long and variable time and then fixed at 70° C. (C). Each of these three sets was divided into two halves, one half was kept for measurement while the other three halves were mixed together and divided into two. One half of the mixture was stained with a 1 :10,000 solution of neutral red for ten minutes as described above (D), while the second half was measured without staining (E). Twenty-five blocks were thus obtained ; 20 apparently sound larvae were measured from each block ; in set D only the unstained larvae were measured. The results, with an

analysis of variance, are set out in Table 9. The analysis of variance over all treatments shows that there is a significant block variance, while the treatment variance is significantly higher than the block variance, thus showing how thoroughly inconsistent are the results generally. Study of the analysis for treatments A and D only, however, will show that for these two sets this is not so. Neither V_2 nor V_3 is significantly

TABLE 9.

Results of staining technique test.

L		A					B					C					D					E				
	604	601	603	604	603	571	577	598	611	592	601	577	559	602	575	617	607	605	616	604	587	602	582	585	590
24	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	1	–	–	–	–
25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
26	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	3	–	1	1	–
27	1	–	–	–	–	2	2	–	–	1	–	2	2	–	7	–	1	–	–	–	5	1	4	1	4
28	1	–	–	–	1	10	6	2	1	4	1	4	10	5	1	1	2	3	–	2	1	3	3	4	1
29	3	6	8	4	5	4	6	4	3	4	4	9	3	1	3	3	1	3	1	3	3	3	3	4	2
30	7	8	6	9	7	3	5	8	5	4	6	5	3	5	4	3	6	5	9	7	5	6	5	6	8
31	6	5	2	6	5	1	1	6	6	7	6	–	–	6	3	6	6	5	6	5	1	3	3	3	4
32	–	1	3	1	1	–	–	–	5	–	1	–	–	2	–	5	3	3	2	3	–	2	–	–	1
33	2	–	1	–	1	–	–	–	–	–	1	–	–	1	1	2	1	1	1	–	–	1	–	–	–
34	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	1	–	–
Sx	604	601	603	604	603	571	577	598	611	592	601	577	559	602	575	617	607	605	616	604	587	602	582	585	590

General Analysis of Variance.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	957·65	475	2·01 (V_1)	—
Blocks	160·36	20	8·018 (V_2)	4·0 (V_2/V_1)
Treatment ...	137·772	4	34·443 (V_3)	17·1 (V_3/V_1)
Total	1255·782	499	—	—

Analysis of Variance on A and D.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	297·7	190	1·563 (V_1)	—
Blocks	7·671	8	0·959 (V_2)	1·64 (V_2/V_3)
Treatment ...	6·149	1	6·149 (V_3)	3·94 (V_3/V_1)
Total	311·520	199	—	—

greater than error. In other words, staining has enabled one to pick out the larvae from treatment A, which had been carefully prepared, when they were mixed with shrunken larvae from treatments B and C, even though this could not be done by eye alone.

It was felt by the authors that this very remarkable result justified the further experiments (mentioned above) performed with a view to eliminating the anomalous results obtained by operators D and F in the main "operator experiment." These further experiments were carried out in exactly the same way as before, except that the larvae were stained after fixing, and only unstained larvae were measured. The new results obtained by D and F were then found to have no significant difference from those obtained by the other operators, and in a new analysis of variance none of the variances was now significantly greater than the error variance, nor was there any significant interaction.

It may therefore be concluded, in view of this encouraging result, that either technique, with the protection afforded by staining, is capable of giving consistent results in the hands of different operators, provided that each operator is prepared to acquire a little practice in its handling.

(*d*) *Time of measurement in relation to liberation and fixing of the larvae.*

During the recovery of larvae from cysts and their preparation for measurement there are three distinct phases: first the period during which they exist in the unhatched eggs set free from the cyst, secondly the period during which they exist as living larvae, and thirdly the period between fixation and measurement. In all three phases the larvae are immersed in distilled water, and during this time they may alter in length. If this is so, it is obviously necessary to determine the extent of the alteration, in order to ascertain, when a given procedure is adopted, the relationship between the measurements made at various times after liberation. Preliminary experiments indicated that a prolongation of the first of these periods to 24 hours had no significant effect on larval length, but that an increase in either of the other two periods resulted in increases in the length of the larvae. In order to understand the problem fully it is necessary to consider it in some detail: suppose the time during which the liberated larvae exist as living organisms be denoted by A, and the time between fixation and measurement by B, it is then necessary to determine the relation of the larval length to any given value of A or B. Moreover, if the length of a larva be denoted by any function of A or B, then it is by no means certain that the function expressing the relationship of length to B will be constant for all values of A, nor that the same function will express its relationship to A, since it is obvious that there must be a limit to the expansion of any larva—a state of affairs which would be impossible were the two functions completely independent. Moreover it is possible that the functions might also vary with the technique used for preparing the larvae. An experiment was accordingly designed in which the effect of varying times both before and after fixation on the length of the larvae was determined both for the hypochlorite and the " squash " techniques.

A series of preliminary experiments had disclosed the fact that if length were expressed as a regression on time of soaking of the larvae in either a living or a fixed condition, then the regression obtained was non-linear, and a graph in which length was plotted against time approximated to a parabola. Further investigations however convinced the authors that for the first eight hours, if length of larva were plotted against log. time, a straight line would give a sufficiently good approximation to the changes actually occurring. A series of experiments was therefore designed with the object of investigating this point.

The time intervals chosen were in geometrical progression, their values being 0·5, 1·0, 2·0, 4·0 and 8·0 hours. Five series of experiments were conducted in each of which the period A had one of these values and measurements were made with the period B equal to 0·5, 1·0, 2·0, 4·0 and 8·0 hours. Two sets of five series were performed, one on larvae obtained by squashing, the other on larvae obtained with the aid of hypochlorite.

Batches of about 500 cysts were taken and the larvae recovered from them by the appropriate technique. The larvae were fixed at intervals after liberation corresponding with the values given to A and samples of 50 larvae were then withdrawn and measured at intervals corresponding to the required values of B. The number 4 eyepiece previously

TABLE 10.

Effect of time B on the length of larvae, for varying values of A ("squash" technique).

	B	½	1	2	4	8				
A	1+log B	0·699	1·000	1·301	1·602	1·903	b	S.E. of b	t	Remarks
½		2630	2650	2669	2720	2697	1·355	0·469	2·96	significant
1		2657	2676	2680	2730	2761	1·712	0·340	5·05	significant
2		2689	2701	2669	2720	2746	0·8837	0·361	2·45	significant
4		2689	2703	2711	2705	2727	0·5422	0·334	1·63	not significant
8		2688	2702	2700	2717	2692	0·3629	0·612	0·539	not significant

referred to was used in this and all succeeding experiments. The measurements of the variations in length of larvae obtained by squashing are given in Table 10. It will be observed that the values for log. time set out in this table are not the true logs. of the times given, but correspond to 1+log. t. If the values of time are examined, it will be seen that the log. of the second value (1) is 0. If this were used as it stands, then in calculating the regressions of length on time all the values of length for this value of time would have to be multiplied by 0 and would consequently disappear. Accordingly 1·00 was added to each value of log. time; the effect of this, obviously, is to convert the time scale from hours into tenths of hours. In this table the individual measurements are not given, due to considerations of space, instead, the sums of the lengths (Sy) are given for each value

of B. Table 11 gives the calculation of the regression of length on log. A. The larvae referred to in this table were fixed at varying times after " hatching " and measured half an hour after fixing (B = 0·5 hr.). With these results have been combined the results from that part of the previous table when B = 0·5.

A study of Tables 10 and 11 discloses the following facts :—the regressions of length on log. B for values of A = 0·5, 1·0 and 2·0 are significant, but where A = 4·0 and 8·0 they are not so. The regression of length on log. A is also significant. It was accordingly decided

TABLE 11.

Effect of time A on the length of the larvae when B = ½ (" squash " technique).

A	½	1	2	4	8	*b*	S.E. of *b*	t	Remarks
1 + log A	0·699	1·000	1·301	1·602	1·903				
	5330	5416	5416	5469	5483	1·193	0·2582	4·62	significant

to compare the regressions on log. B with one another. When this was done a mean variance of 0·1583 was found to apply to the statistic *b*, giving a value of 0·3166 for the variance of the difference between any two estimates of this statistic. As N is 744, the values of t for significance will be 1·95996 for p = ·05 and 2·57582 for p = ·01. The critical difference for significance will therefore be 1·107 for p = ·05 and 1·448 for p = ·01. Since the maximum difference between values is 0·828, it can be said that all the values of *b* for values of A = 0·5, 1·0 and 2·0 are different estimates of the same parameter, and can therefore be combined to give a single estimate of *b*. Summing the covariances over these three values of A, a figure of 178·998 is obtained, while $S(x-\bar{x})^2 = 135{\cdot}009$, giving a general regression of 1·326 for length on log. B. The regression of length on log. time is therefore 1·326 scale divisions per unit increase in log. time, i.e. 22·187μ, when the time interval is from fixing to measurement. This regression applies in all cases where the larvae are fixed less than two hours after hatching.

A comparison between the regression of length on log. A and the mean regression of length on log. B was next made. The variance of the difference was found to be 0·11433, and the standard error is therefore 0·3381 ; since the arithmetical difference is 0·133, a value of 0·3933 is obtained for t, which is thus not significant. Accordingly, the lumping of regressions was carried a stage further and a single

regression was obtained from all the regressions so far calculated. The value of this is 1·2723 with a standard error of 0·1658, corresponding to an increase of 21·288μ per unit increase in log. time.

The results to date may accordingly be summarised by saying that, if the larvae are fixed less than two hours after hatching, they increase in length to a significant degree the longer the delay between fixing and measurement. The rate of this increase, if plotted against log. time, is constant for eight hours, and for varying times between " hatching " and fixing; moreover this rate of increase is the same as the rate applicable to the increases in the length of larvae when they are soaked

TABLE 12.

Effect of time B on the length of larvae for varying values of A (hypochlorite technique).

A	B	½	1	2	4	8	b	S.E. of b	t	Remarks
	1+log B	0·699	1·000	1·301	1·602	1·903				
½		2656	2686	2634	2692	2674	+0·279	0·37	0·754	not significant
1		2675	2683	2691	2664	2687	+0·033	0·348	0·0963	not significant
2		2684	2693	2675	2640	2666	−0·59	0·366	1·63	not significant
4		2672	2679	2619	2607	2446	−3·48	0·416	8·37	very significant
8		2574	2614	2583	2524	2534	−1·129	0·455	2·49	significant

before fixing. If the larvae are soaked for more than two hours, however, the regression of length on log. time between fixing and measuring is no longer significant, i.e. the larvae no longer swell after fixing although swelling occurs over the whole period prior to fixing. These results apply to larvae obtained by the " squash " technique.

The results of a similar series of experiments on larvae obtained by the hypochlorite technique are set out in Table 12. It will be seen that the only significant regressions are those corresponding to A = 4 and 8 hours. These two regressions are significant but negative in sign, in other words they afford evidence of shrinkage of the larvae. The value for the regression coefficient for A_4, however, is suspect, since the high figure computed for it is to a very great extent due to the

very low value in the last series of measurements (B_8). The lack of significance in the bulk of the regressions is further emphasised if an analysis of variance be performed on the data. It is not possible to publish the analysis, but it was found that for values of A = 0·5, 1 and 2 hours no significant difference could be detected between the lengths at different times, but for values of A = 4 and 8 hours there was a difference, the later figures in each series tending to be shorter than the earlier. In order to determine the effect of time before fixing on the length of the larvae, a calculation was performed on the values appropriate to B = 0·5. It was then found that there was no significant difference between any of the first four values, but that the fifth value was significantly lower than the rest. It may therefore be concluded that larvae recovered by means of the hypochlorite technique do not alter in length after " hatching " provided that they are not kept longer than two hours before fixing : after fixing no significant increase in length occurs. It is important to realise, however, that in these experiments no information has been collected as to what alterations in length occur during the first half hour after " hatching " or fixing. It is not at all unreasonable to expect that any changes in length that may occur would take place at a far greater rate during this period than during a later period, and it would be distinctly unsafe to assume that the regressions and relationships derived above would necessarily hold for periods within the first half hour.

Bearing in mind the above facts, conditions may be laid down regarding the best time for measuring larvae. It has been shown that the swelling of larvae obtained by squashing occurs at a constant rate when plotted against log. time, whether the swelling takes place before or after fixing. This means that if the total time between " hatching " and measuring be reasonably short (say, 0·5 to 1 hour), it is unnecessary to pay great attention to the time of fixing provided that the time from " hatching " to fixing is not vastly different from that between fixing and measuring. It has been shown that when the larvae are measured 30 minutes after " hatching," the same results are obtained whichever way they have been recovered. It is accordingly recommended that larvae be measured 30 minutes after liberation. Should measurements be made at any other interval, it is distinctly advantageous that the exact intervals between liberation, fixing and measuring be stated, in order that the results may be capable of " standardization " to periods of 30 minutes by means of the regres-

sions given by the authors. Measurements made at unstated intervals of time are valueless, since they are incapable of comparison with other results. A last condition to be laid down is that there should not be more than two hours delay between liberation and fixing of larvae which are to be measured, since even if the correct times are stated, the regressions given above do not apply, and the standardization of data obtained from such larvae to the conditions stipulated above would, to say the least of it, involve a very complicated computation.

III. Choice of the Sample of Larvae to be Measured and Interpretation of the Results.

In determining parameters for the length distribution of any species of eelworm larva it is obviously necessary that the parameters derived should be the very best estimates that it is possible to obtain. As far as possible they should be universal in their application, and all the errors to which they are subject should be thoroughly investigated. After a perusal of the literature recording larval measurements, the authors feel that too little attention has been paid to the specification of the sample from which the larvae have been derived. A *sine qua non* of sampling practice, whatever the substance being sampled, is that the sample should be a representative cross-section of the whole being examined, and not merely a portion from one part of it. Important as is the procedure of sampling in ordinary and comparatively simple problems like the sampling of the soil in a field, it is of even greater importance when one is concerned with the very complicated system presented by a population of eelworm cysts. In the first case one is dealing with a phase which is distributed more or less unevenly through another and different phase, whereas in the latter one is dealing with a population that is naturally made up into "parcels." A sample of such a heterogeneous population must reflect not only the state of affairs over the whole population generally, but also the state of affairs within each "parcel." In the case of *Heterodera* spp. (excluding *H. marioni*) this "parcelling" appears to have been carried by nature to a remarkable degree. Let us consider, for example, how a population of *H. rostochiensis* is built up. In the first place the larvae are grouped into obvious small parcels, the cysts; the latter are themselves less obviously grouped according to the host on which they were formed, cysts from one plant constituting one parcel, those from another plant a different parcel; the next higher group depends upon

the variety of the host, e.g. the potato may be an early or a maincrop variety; there may be several varieties in a host species, and several species in the host range of the eelworm in question, e.g potatoes and tomatoes in the case of *H. rostochiensis*. Host species may further be grouped according to the locality in which they were grown (e.g. tomatoes grown in the open in Jersey and under glass in Yorkshire may both be infected with *H. rostochiensis*). Any one or all of these factors may have a significant effect on the length of the larvae, and before a technique for sampling can be laid down it is necessary that each be examined and investigated. It is felt that in the past this subject has not had the attention that it deserves; in many cases all that is given is a figure for the mean length of a number of larvae—a constant which is quite useless if it is unaccompanied by information regarding the variations in length normally encountered within a population. Only slightly more valuable are results which give the mean of a population together with a standard deviation, without in any way specifying the sample from which the figures were derived, since without this information the applicability of such parameters is nil, in view of the fact that where one has a " parcelled " population any one of a number of standard deviations may apply to a sample, depending on its nature. Suppose, for example, that one has measured a sample of potato eelworm larvae, obtained from a number of cysts derived from a number of plants, and that the source of each larva measured can be traced to a particular cyst from a particular plant. If an analysis of variance be performed on the data obtained in will disclose three main sources of variance; error, the variance of cyst means within plants and the variance of the plant means, as well as the total variance of all the larvae. It is an interesting fact, however, as will be shown in a later stage of this paper, that none of these variances, except possibly the error, is in any way universal in its application; each is itself governed by the nature of the sample and the size of each of its consistent parts. Thus, if the sample is made up of ten larvae per cyst from each of ten cysts per plant from each of ten plants, the " between-plant " variance would be different from that for a sample derived from three larvae per cyst, twenty cysts per plant and ten plants. The total variance and the " between-cyst " variance would also differ, since it can be shown that each of the higher orders of variance is itself made up of a portion ascribable to itself and also to portions ascribable to the lower orders of variance. To determine

the relative importance of all these factors it is necessary to determine the magnitude of each of these sources of variation.

An experiment with this object was accordingly performed on the potato eelworm. Ten cysts were removed from the roots of each of five plants belonging to each of seven varieties of potato which had been grown together on an infected plot of land. The varieties concerned were all maincrop varieties, *viz.*, Arran Banner, Great Scot, Majestic, King George, Doon Star, Kerr's Pink and Golden Wonder. The cysts from each of the 35 plants were stored separately in a dry state. Before use they were soaked for several days in distilled water and the larvae were then recovered from each

TABLE 13.

Analysis of Variance on lengths of larvae derived from different cysts, plants and varieties.

Source	Sum of Squares	D. of F.	Variance	Variance ratio
Error	12,563·600	3150	3·988 (V_1)	—
Cysts	6,634·660	315	21·0624 (V_2)	5·27 (V_2/V_1)
Plants	1,696·188	28	60·578 (V_3)	16·48 (V_3/V_1) 2·89 (V_3/V_2)
Varieties... ...	278·295	6	46·3825 (V_4)	11·62 (V_4/V_1) 2·21 (V_4/V_2)
Total	21,172·743	3499	6·051	—

cyst by the "squash" technique. Ten larvae were measured from each cyst, giving a total of 3,500 measurements. An analysis of variance of the data thus obtained is given in Table 13. A comparison of the variances discloses the fact that the "cysts-within-plants" variance is significantly greater than the error variance, while the "plants-within-variety" is greater than either. The "between-variety" variance is not greater than the plant variance within variety. This result at once furnishes valuable information which is of great use when deciding on the size and form of the sample which must be taken in order to obtain reliable parameters for this species of eelworm. The ideal is obviously that an infinitely large number of larvae be examined from each cyst, an infinitely large number of cysts per plant, and so on; but, admirable though such a procedure might be in theory, it would obviously be quite impossible in practice: one can

but make the best use of the necessarily restricted material available. If it is desired that the final sample shall consist of 1,000 larvae, how is this sample to be made up? In the first place, the variations due to variety of host, at least in the case of the seven varieties tested, are not greater than those due to plants, and the sub-division of plant species into varieties is, in this case, therefore, not of paramount importance. In any case, the number of varieties available will probably be limited, and the best procedure will be to examine larvae from plants of all possible varieties and, when analysing the data, merely to compute the variance due to plants generally. Since the variance due to plants is greater than that due to cysts, attention must be paid to the ratio of cysts to plants, and where a choice has to be made it is better to choose a small number of cysts from each of a large number of plants than vice versa. The same principle should be applied to the choice of the number of larvae to be measured from each cyst; the number of cysts should always be increased at the expense of the number of larvae per cyst. From a statistical point of view the reduction in the number of larvae measured per cyst whilst the number of cysts is increased, and the similar reduction in the number of the cysts whilst the number of plants from which they are obtained is increased, does not result in a great loss in the number of degrees of freedom of the lower variances. Suppose a sample of 1,000 larvae be made up of 20 larvae per cyst, 10 cysts per plant and 5 plants, then the number of degrees of freedom for error will be 950, for cysts within plants there will be 45 and for plants 4. It is obviously desirable to increase the number of degrees of freedom between plants, and also, if possible, between cysts within plants. Let this sample now be made up of 5 larvae per cyst, 20 cysts per plant and 10 plants, then error variance will be based on 800 degrees of freedom, " cysts-within-plants " on 190, and " between-plant " variance on 9. The number of degrees of freedom on which " between-plant " variance is based has been more than doubled, that for " cysts-within-plants " quadrupled, while the reduction in the number of degrees of freedom for error amounts to about 17%, and the analysis of variance is better balanced.

A further point arises in the handling and interpretation of the data derived from the analysis of variance. It is obvious that what is required is information applicable to the whole population of which the sample is but a part, and care has to be taken to see that parameters for the sample are universal in their application. Consider the problems

which the authors are attempting to elucidate; in the first place it is desired to identify a single cyst on the basis of the length of the contained larvae, and secondly to determine the relative levels of each of several infections present in a soil. To deal with the first problem first; in Table 13, V_3 and V_4, not being significantly different from one another, may be pooled, giving a variance of 58·1 for plant totals generally. Since each plant total is derived from 100 values, this variance will be 100 times the variance of plant means, the true value for which will therefore be $0{\cdot}581 = V_p$, say. V_p is an estimate of the variance of plant means when each plant mean is based on measurements of 10 larvae from each of 10 cysts per plant. It can be proved that this value, of 0·581, is itself not a simple variance, but is composed of two parts, *viz.* the variance of plant means if each mean were derived from an infinite number of measurements of larvae per cyst, and an infinite number of cysts, plus the error to which each plant mean is subject as a result of the mean actually being derived from 10 cysts and 10 larvae. With regard to the latter of these components, each plant mean is derived from a series of 10 cyst means, there being 35 of these series contributing to the 35 plant means. The mean variance of these series is $V_2/10 = 2{\cdot}106$, and the variance of an estimate of each plant mean will therefore be $2{\cdot}016/10$ $(= V_2/100)$. Let the first component be represented by σ^2_p, then $V_p = \sigma^2_p + V_2/100$, whence $\sigma^2_p = V_p - V_2/100 = 0{\cdot}3704$. This is an estimate, based on 34 degrees of freedom, of the variance of plant means when each mean is derived from measurements of an infinite number of larvae from an infinite number of cysts. Now consider the case of cysts found in the soil; to all intents and purposes they are all unrelated to one another and no two in a sample need necessarily have come from the same plant. Suppose an infinite number of larvae are measured per cyst, let the variance of the means per cyst be σ^2_c. Here we are dealing with a population of "plant means," each mean being derived from one cyst, so that, if the variance of cyst means within plants when an infinite number of larvae be measured per cyst be represented by V , then the variance of the "plant means" (which will be cyst means as well in this case), represented by V, will be the sum of σ^2_p + the variance of cyst means within plants, i.e. $V = \sigma^2_p + V_c$. It is now necessary to derive V_c. This quantity may be derived in a manner similar to that used for deriving σ^2_p. In Table 13 V_1 is the variance of larvae within cysts and V_2 the variance of cyst means x 10; the latter is itself made up of two

parts, V_c and the variance of each estimate of each cyst mean; this latter $= V_1/10 = 0{\cdot}398$, and $V_2/10$ may therefore be equated to $V_1/10 + V_c$, whence $V_c = V_2/10 - V_1/10$. This may now be substituted in the equation for V and the value of V is found to be $\sigma^2_p + V_2/10 - V_1/10$: the latter is an estimate of the variance of cyst means when each cyst comes from a different plant, and when an infinite number of larvae are measured per cyst. However, should only a limited number of larvae be measured, then each cyst mean would itself be subject to a standard error of $\sqrt{V_1/n}$, where n is the number of larvae measured per cyst. Thus the variance of the cyst means would be $\sigma^2_p + V_2/10 - V_1/10 + V_1/n$. σ^2_p has already been shown to be $V_p - V_2/100 = 0{\cdot}3704$. Hence the variance of cyst means generally, when n larvae per cyst are measured, is given by the formula $V = V_p - V_2/100 + V_2/10 - V_1/10 + V_1/n = V_p + \frac{9V_2}{100} - \left(\frac{n-10}{10n}\right) V_1$. Using this formula it is possible to calculate the variance of cyst means for the species under discussion, and for any number of larvae measured per cyst. Should 10 larvae be measured, then the last term $\left(\frac{n-10}{10n}\right) V_1$, becomes zero, and a value of 2·476 is obtained from the variance, corresponding to a standard deviation of 1·573. If 50 larvae are measured the variance will be 2·1573, and the standard deviation is 1·47, while if an infinite number is measured per cyst the variance will be 2·077, and the standard deviation is 1·442. It should be noted that should fewer than 10 larvae be measured (n–10) becomes negative, and the last term must therefore be added to the general formula, not subtracted. Another point worthy of interest is that the figures just given refer to the variance of different cyst means, they do not refer to the variance of the figures obtained for any one cyst, which will, of course, be an estimate of V_1 in the analysis of variance. It will also be noted that it is not necessary to examine many larvae per cyst in order to identify the cyst, since the figures given above show very strikingly how small is the increase in accuracy obtained when the number of larvae measured is increased above 10; it is suggested that 10 or 20 would be a suitable number to measure. If 20 larvae per cyst are measured, the variance of cyst means would be 2·2767, with a standard deviation of 1·51. In absolute units this equals 13·98μ. The grand mean for all larvae in this experiment was (51·406 − 0·5) divisions, when the correction for grouping has been applied, and this = 471·39μ.

Limits can therefore be laid down for the variations in mean length of 20 larvae from a single cyst of potato eelworm, based on the lengths given by 3,500 larvae derived from 350 cysts from 35 plants of 7 varieties of potato grown simultaneously on a small area of land. 95% of such cyst means will lie between $471{\cdot}39 \pm 28\mu$, i.e. between $443{\cdot}39$ and $499{\cdot}39\mu$. This technique for deriving parameters will be used by the authors in future work dealing with the identification of single eelworm cysts by means of larval measurements.

In order to settle the second problem, that of determining the relative levels of each of several eelworm infections present in a soil, it is first necessary to estimate as accurately as possible the parameters descriptive of the length distribution of the larvae of each species generally, i.e. to determine these parameters for samples of completely randomised larvae of the given species. It is hoped to discuss in a later paper the use of these figures in the differentiation of *Heterodera* species. When a sample of larvae is obtained from the soil the source of any one of them is generally unknown, and one must therefore assume that each one may have a different origin from all the others. The best estimate of their parameters is one based on a sample in which no two larvae are derived either from the same plant or from the same cyst. In other words the parameters are computed for a sample derived by measuring one larva per cyst from one cyst per plant, etc. In practice to measure such a sample would involve an unreasonable amount of work, but reliable estimates of these parameters may be arrived at by computation of data given in the analysis of variance in Table 13. The variance of plant means (V_p) has been shown to be 0·581, and a value was computed for the variance of cyst means generally for any given number of measurements of larvae per cyst. The variance of cyst means has been shown to be $V = V_p + \frac{9V_2}{100} - \left(\frac{n-10}{10n}\right) V_1$.

Hence, when n is infinity $V = V_p + \frac{9V_2}{100} - \frac{V_1}{10}$. Suppose only one larva per cyst had been measured, each cyst mean would be subject to a standard error of $\sqrt{3{\cdot}98}$, and the variance of the final series would be the sum of this plus V. Thus, if the variance of a sample of larvae all derived from different sources be represented by σ^2_L, then $\sigma^2_L = V_p + \frac{9V_2}{100} - \frac{V_1}{10} + V_1 = V_p - \frac{9V_2}{100} - \frac{9V_1}{10}$ and substituting the appropriate values from Table 13 a value of 6·065 is obtained

for the variance of such a series, giving a standard deviation of 2·46 divisions, i.e. 22·79μ. A figure obtained in this manner is universal in its application and relates directly to the distribution of larvae generally. It is the figure to be used in determining the species of any larvae of unknown origin. It is hoped to describe in a later paper the use of parameters obtained in this way.

A final point for discussion is the nature of the distribution of lengths characteristic of eelworm larvae. The technique proposed by the authors (to be described in a future paper) depends largely on the normality of this distribution. Tests were therefore performed to determine whether or not this distribution is in fact normal. The larvae were recovered from ten cysts, and 100 larvae from each cyst were measured. A χ^2 test was made on each of these ten populations and the ten χ^2 results were added together, also the total number of degrees of freedom appropriate to each. A value of 30·1538 was obtained for χ^2, with 23 degrees of freedom, which gives 1·07 for the expression $\sqrt{2\chi^2} - \sqrt{2n-1}$, corresponding to a probability of 0·28 – a perfectly satisfactory result. It is therefore reasonable to assume that the distribution of larval lengths within cysts is normal. The distribution of cyst means within plants was next tested, the data from Table 13 being used for this purpose: the result was a value for χ^2 of 6·946 based on 9 degrees of freedom and with a probability of between 0·70 and 0·50. A further test of the distributions of cyst means generally gave a χ^2 of 8·10394 based on 9 degrees of freedom and with a probability of between 0·70 and 0·50. A test was then made of the distribution of plant means. This test was not altogether satisfactory because there were only 35 values, and χ^2 had therefore to be based on very few degrees of freedom. The value obtained was 3·713 based on 3 degrees of freedom, with a probability of between 0·30 and 0·20. One may therefore conclude that, in considering larval lengths, one is dealing with normal distributions, and that therefore the usual tables for areas of tails and normal ordinates are applicable to the problems relating to *Heterodera* larvae.

It must be borne in mind that the actual values given above for parameters of the length distribution of larvae of *H. rostochiensis*, and the conclusions arrived at with regard to sampling practice, are based on material from only seven varieties of potato, all maincrop varieties and all grown in one locality under the same conditions. It is possible that the conclusions may have to be modified when larvae from other varieties and species of host and from other localities come to be

considered. The sampling investigations herein recorded represent merely preliminary work on sampling problems. They are recorded only in order to show the complexities of the problem, and to indicate in a tentative manner the type of information required and how it might be obtained and analysed, in order to obtain reliable and universally applicable estimates of the parameters of larval lengths for a given species of *Heterodera*.

Summary of Conclusions.

1. *Sampling*.

A complete investigation of the variations likely to occur in the lengths of larvae of *Heterodera rostochiensis* (for example) under all conditions would involve measuring larvae from a great number of varieties of potato grown in a great number of different localities, and also of larvae from tomatoes of different varieties grown under various conditions. The results obtained so far for this species of *Heterodera* make but a small contribution towards the mass of information which might be accumulated. Until such further data become available, certain conclusions have been drawn as to the best method of taking samples of larvae for measurement, but it must be remembered that these conclusions may need revision at a later date in the light of new information. According to our present knowledge it appears that the variety of potato on which a cyst has developed does not significantly affect the mean length of the larvae within, but that the mean length of the larvae from a single plant, irrespective of the variety, may differ significantly from that of larvae from another plant. It is also deduced that the variations in the mean lengths of the larvae from different cysts are greater than the variations within the individual cysts. From these facts it follows that if one wishes to estimate parameters for larval lengths of a population of *H. rostochiensis* larvae, a better estimate will be obtained if one takes a small number of larvae from each of a large number of cysts rather than the same total number of larvae from a small number of cysts. Similarly the cysts should have come from a large rather than a small number of plants, but apparently it is not necessary that the plants should be of as many varieties as possible, though this last assumption is based on information derived from only seven varieties of potato, all of them maincrop varieties, and it may need modification when larvae from other varieties have been examined.

In addition to this information as to the best method of obtaining a sample of larvae, values for parameters applicable to the larvae measured in the experiments have been computed, and the methods of computation are given. The mean lengths of 20 larvae from single cysts of *H. rostochiensis* from the potato, based on measurements of 20 larvae from each of ten cysts from each of five plants from each of seven varieties of potato (=3,500 larvae) were such that in 95% of cases they fluctuated between 499·39 and 443·39μ. A further computation is given showing that for a sample of such larvae, each of which came from a different cyst and each cyst from a different plant, the standard deviation would be 22·79μ, i.e. in 95% of cases the lengths would lie between 425·39 and 517·39μ. This figure represents the best estimate that can be given at present of the mean length of larvae of *H. rostochiensis* prepared and measured in the manner described in this paper, and it may be used in comparing larvae of this species with those of other species of which the parameters have been similarly derived. Finally, it has been proved statistically that the distribution of lengths of larvae within individual cysts is normal, and that the distribution of the means of larval lengths for the cysts from a single plant (i.e. cyst means " within plants ") is also normal. It may therefore be assumed that larval lengths in general are normally distributed, and that the usual statistical procedure for such distributions may be adopted.

2. *Preparation of larvae for measurement.*

The experiments described have brought out very clearly several points of paramount importance in the preparation of larvae for measuring. Firstly, the temperature used for fixing the larvae must not be over 80° C., or shrinkage will take place. The usual practice, when it has been necessary to kill a nematode for microscopic examination, has been to warm the drop of water containing the nematode on the slide over a low flame until the nematode has appeared dead. While this method, when carried out carefully, may give perfect results, it is extremely easy to allow the water to become too hot, and although actual boiling may be avoided it is easily possible to heat the nematode above 80° C. and cause shrinkage. In the second place it has been shown that the lapse of time between " hatching," i.e. emergence of the larva from the egg, and measuring of the larvae must be carefully controlled, since larvae soaking in distilled water gain considerably in length both before and after fixing, if they have been liberated

mechanically, while if they have been liberated by means of calcium hypochlorite solution they may shrink if there is a delay of more than six hours before they are measured. Thirdly, care must be taken that the person measuring the larvae has had enough practice and experience to enable him to obtain reliable results. And lastly, only sound, undamaged, and of course fully grown larvae must be measured. Besides taking into account these important points, a reliable technique for obtaining the large numbers of larvae necessary for determining the parameters of their length-distribution should liberate most of the larvae contained in the cysts rapidly and in good condition : it should be simple and enable large numbers of larvae to be measured quickly, and it should include a method of enabling the inexperienced worker to distinguish between sound and damaged larvae.

As a result of the information gained from the experiments described in Part II of this paper the following conditions have been adopted for the preparation of larvae to be measured. The actual liberation of the larvae from the eggs may be brought about either by pressure on the eggs or by means of a solution of calcium hypochlorite. Whichever method is used the cysts must first be soaked in distilled water for 3 or 4 days, or at least until they sink to the bottom of the vessel. The sample of cysts is then placed in a drop of distilled water on a slide, a second slide is placed on top, and by means of pressure and lateral movements of the upper slide on the lower the cysts are broken and the contained eggs liberated and mixed. The eggs are then removed by means of a fine pipette to the vessel or slide where the larvae are to be extracted. If the hypochlorite technique is to be used the eggs are collected in as small a drop of water as possible in a solid watch-glass and 1–2 cc. of calcium hypochlorite solution added. This solution is very unstable and must therefore be made up freshly for each experiment from a stock solution. The stock is a 5% solution of B.D.H. bleaching powder in distilled water : this will contain about 1% available chlorine, but this point should be tested as described by Fenwick (1942). It will keep fairly well for about 14 days in a well stoppered bottle. The solution used to liberate the larvae from the eggs is prepared by adding 7·5 cc. of the stock solution to 50 cc. of distilled water, and contains about 0·126% available chlorine. After 10 minutes in this solution, when most of the larvae will have " hatched " from the eggs, the hypochlorite solution is withdrawn and the watchglass is filled with distilled water. The water is changed several times in order to remove

all the hypochlorite, care being taken to allow the larvae to settle before the water is drawn off, so that as few as possible of the larvae are lost. The last lot of water added is at a temperature of 75–80° C., and fixes the larvae. If the " squash " technique has been chosen, the liberated eggs are placed on a slide instead of in a watchglass, and all surplus water is removed. A large coverslip is placed on the drop and gently pressed with a blunt instrument such as the handle of a fine paint brush. This is done under the binocular microscope so that it can be seen when the pressure is sufficient to burst the eggs without damaging the larvae. When most of the larvae have been liberated in this way the coverslip is removed and the larvae are washed off the slide and coverslip into a solid watchglass by means of a fine jet of water. They are left to settle and most of the water is then withdrawn. The watchglass is filled with water at 75–80° C. to fix the larvae as in the hypochlorite technique. In both techniques it is imperative to observe the time at which most of the larvae are first freed. After the larvae have been fixed in the hot water the two techniques are identical. The larvae are again left to settle and the water is replaced by a 1 :10,000 solution of neutral red, in which they are left for at least 10 minutes. If, in the case of the hypochlorite technique, the stain loses colour, this indicates the presence of hypochlorite, and the larvae must be washed again until it is all removed and the neutral red is no longer bleached. The larvae are concentrated to the centre of the vessel by shaking it with a circular motion and a small drop of stain with as many larvae as possible is drawn up in a pipette and transferred to the cavity of the measuring slide described in Part I. The cavity is filled with distilled water, thus diluting the stain sufficiently for the purpose of measuring the larvae, the latter are allowed to settle and surplus water is withdrawn till the surface is flat. When 30 minutes have elapsed from the time when the larvae were first liberated they are ready to be measured.

Either of the above techniques may be used for obtaining larvae either from a bulk sample of cysts or from single cysts, but if larvae from a single cyst have to be measured it will probably be quicker to use a modified form of the " squash " method, as this eliminates the 10 minutes wait while the hypochlorite is acting on the eggs. It was found convenient to place the cyst from which larvae are to be freed in a very small drop of water in a solid watchglass. The watchglass is placed on the stage of the binocular microscope and first the cysts,

then the eggs are gently squashed under a specially made glass "pestle." This is in the form of a small spatula made from a piece of glass rod of 5 mm. diameter and 12 cms. long; one end of the rod is flattened into a spatula not more than 0·5 mm. thick, and bent at an angle of about 130° with the neck. With this instrument it is quite easy to exert gentle pressure on individual eggs, and the spatula is thin enough for the process of squashing the eggs to be seen through it, so that damage to the larva can be avoided. With a little practice 20 eggs can be broken and the larvae liberated without damage within a minute. When the required number of larvae has been liberated they are fixed by filling the watchglass with water at 75–80° C. in the usual way, then stained so that damaged or overheated larvae, if present, may be picked out, and the sound larvae are measured 30 minutes from the time when they were liberated.

To sum up it may be said that the hypochlorite technique has the great advantage that almost all larvae are liberated in an unsquashed condition, which may be of considerable importance in cysts from soil samples many of which may be more or less empty, and in which the number of available larvae may therefore be restricted. In addition, during the period between half an hour and two hours after "hatching" the larvae do not alter in length, so that fixing and measuring may be carried out at any time during this period, whereas when the larvae are liberated by squashing the eggs there is a constant rate of increase in length up to eight hours from liberation.

3. *Measuring of the larvae.*

The most efficient way of measuring large numbers of eelworm larvae was found to be by measuring to whole divisions on a micrometer eyepiece scale. A satisfactory combination of lenses was a x6 eyepiece with a number 4 objective, which gave an image of the larva extending along about half the micrometer scale. The convention was adopted of recording measurements which were not exact whole numbers as the next higher number of divisions above that just exceeded. The grouping of the values thus brought about does not affect their statistical treatment, but to arrive at the true value of a measurement or a mean in the units employed (i.e. in numbers of divisions) 0·5 division must be subtracted from the recorded figure.

Since the larvae could not easily be arranged under a coverslip, it was found necessary to mount them in a large drop of water with a

flat surface in the cavity of a specially prepared slide (described earlier) so as to avoid distortion caused by the variable convexity of the surface of small drops of water. This procedure was rapid and gave results strictly comparable with those obtained when a coverslip was used: in addition, similar results were readily obtainable by different operators using the same method.

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Infestation with Liver Fluke among 73,000 Cattle slaughtered in Great Britain during June, 1942.

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Attention having been drawn by Professor R. T. Leiper to the frequent occurrence of liver fluke in cattle slaughtered for food and to the probability that apart from the consequent economic loss, infected cattle may act as reservoirs, hitherto overlooked in measures advocated for the control of fluke in sheep in this country, the Agricultural Research Council called a conference in March, 1942, to consider the infestation of cattle with liver fluke. This conference was attended by representatives of the Ministry of Agriculture and Fisheries, the Ministry of Food, and research Institutes interested in the problem. It was felt that there was a large but not accurately assessed economic loss, particularly harmful in war-time, due to the partial or total condemnation of cattle livers infested with fluke. The present control of abattoirs by the Ministry of Food made it theoretically possible to check the numbers of fluke-infested cattle passing through the abattoirs and, furthermore, the recording system in use enabled infested cattle to be traced back from the abattoir to the Collecting Centre from which the cattle came. In these circumstances the Conference accepted an offer by Mr. A. W. Anderson (Ministry of Food) to draft a questionnaire, in consultation with the Ministry of Agriculture and Fisheries, for circulation to abattoirs in Great Britain. This form, as finally approved, asked for a return of the numbers of cattle slaughtered at each abattoir during each of the three weeks ending 5th, 12th and 19th June, 1942, the total numbers to be dissected under the following heads :—

A. *Class* : 1, Steers, heifers and cow heifers; 2, Cows; 3, Bulls.

B. *Origin* : 1, Home-bred; 2, Imported; 3, of Unknown origin.

C. *Degree of Fluke Infestation* : 1, No visible evidence of fluke; 2, Liver affected with fluke but not condemned; 3, Liver affected with fluke causing partial condemnation; 4, Liver affected with fluke causing total condemnation.

The forms also asked for the names of all collecting centres from which cattle were received in each of the three weeks, and invited

remarks. It was to be signed by the Slaughterhouse Manager and by the Meat Inspector.

Of the 510 forms returned to the Ministry of Food and forwarded by Mr. Anderson through the Agricultural Research Council, 24 were discarded as being incomplete or ambiguous. The remaining 486 forms contained a classification of 73,372 cattle slaughtered during the three-weekly period, and these constitute the principal data of the present report. There are in addition some supplementary data from a few centres based on periods much longer than these three weeks and in one case (Edinburgh) extending back to 1925 : these supplementary data will be kept separate from the main survey. The latter constitutes, in effect, a vast five-dimensional contingency table, the dimensions being as follows (with the number of classes given in parentheses) : Time (3 weeks), Place (486), Class (3), Origin (3), Degree of Infestation (4). The product of the classes gives the number of cells in the table : 52,488, which is a number comparable with the total number of cattle, a situation in conformity with the fact that there is a high proportion of cells with zero frequency. If the trees are not to obscure the wood, a considerable simplification of this table will be necessary. Moreover, each of the above dimensions requires some explanatory discussion. Simplification and discussion will therefore be dealt with first.

Time.

It was expected that the data for the three separate weeks would show only random fluctuations in the numbers of cattle slaughtered and the proportion found to be flukey, in which case it would be justifiable to take into consideration only the totals for the three-weekly period. The actual totals of slaughtered cattle were as follows :—

1st week : 27,864 or 37·976% of the grand total ; S.E. 0·1792%.
2nd week : 24,096 or 32·841% of the grand total ; S.E. 0·1734%.
3rd week : 21,412 or 29·183% of the grand total ; S.E. 0·1678%.

The expected percentage would be 33·333 with a Standard Error of 0·1740%, so that the S.E. of the difference between any one weekly percentage and the expected 33·333% would be 0·2461%. Hence all these differences are highly significant and cannot be regarded as the results of random variation.

When the infestation rates for each week are considered it can be seen that they are increasing though the absolute numbers are

decreasing. Thus the total numbers of flukey cattle (infestation groups 1, 2 and 3) are as follows :—

1st week : 4,665, or 16·742% of the weekly total ; S.E. 0·2237%.
2nd week : 4,172, or 17·314% of the weekly total ; S.E. 0·2437%.
3rd week : 4,126, or 19·270% of the weekly total ; S.E. 0·2695%.

Comparison of the first and second weeks shows that the difference between the infestation rates is $0·572 \pm 0·3305$ per cent., which is not significant, The difference between the second and third weeks is $1·956 \pm 0·3626\%$, which is highly significant, and so *a fortiori,* is that between the first and third weeks. Thus there is an increased proportion of flukey cattle in the third week. Examination of the details shows that the increase is shared by the "home-bred" and "unknown" groups, the increase occurring in both in the third week only, and being especially marked (7·3%) amongst the unknown. Now, Glasgow accounts for no less than 71·7% of all the cattle of unknown origin, and the Glasgow form shows that there is a sharp rise in the incidence of flukey cattle from the second to third weeks : $9·64 \pm 0·61\%$ to $16·6 \pm 1·01\%$, the absolute numbers of flukey cattle are about the same (227, 237, 226), the totals falling rapidly (2,735, 2,459, 1,362). Moreover, the incidence values at Glasgow are well below the averages for the whole country, especially among the class "Steers, etc.," which constitutes the majority, and especially during the first two weeks. It appears, therefore, that Glasgow had access to unusually clean steers during the first two weeks, when the incidence of fluke in steers was $6·94 \pm 0·52\%$ and $9·55 \pm 0·64\%$ respectively, but that these were largely absent in the third week when $14·3 \pm 0·96\%$ of steers were flukey. This appears to be the explanation of the increase in the proportion of flukey livers in cattle of unknown origin.

A somewhat similar state of affairs exists amongst home-bred cattle over the country as a whole. The total numbers slaughtered are falling (20,941, 18,219, 17,035) but expressed as percentages of slaughtered cattle of all origins ($75·2 \pm 0·26\%$; $75·6 \pm 0·26\%$; $79·6 \pm 0·28\%$) the home-breds constitute a higher proportion of the total in the third week. The numbers of flukey home-bred cattle are fairly steady (3,143, 2,867, 2,965) but expressed as percentages of flukey cattle of all origins ($67·4 \pm 0·70\%$; $68·7 \pm 0·73\%$; $71·9 \pm 0·71\%$) the third percentage is significantly higher than the second. Expressed as percentages of all home-bred cattle slaughtered each week ($15·0 \pm 0·25\%$; $15·7 \pm 0·27\%$;

17·4 ± 0·29%), the third week again shows a significant rise. As to area, both the percentages and the absolute numbers of flukey cattle decline, from the first week to the third, in areas 1 and 3, but the percentages rise in areas 2 (includes Glasgow), 4 and 5, the net effect being the rise under discussion. Thus, as among the Glasgow cattle of unknown origin, the home-bred cattle show an increasing incidence of fluke due, not to an absolute increase in the numbers of flukey cattle, but to a falling off in the numbers of relatively clean cattle.

Strictly, this heterogeneity in the weekly numbers of flukey and of all slaughtered cattle makes it inadvisable to use the three-weekly totals, disregarding variations from week to week. Nevertheless, the effects of heterogeneity are not large in relation to the major comparisons which it is desired to draw and, in order to avoid undue complexity, the succeeding sections will in fact ignore the weekly variation and be based on three-weekly totals.

Place.

Any one abattoir draws its cattle in part from neighbouring villages, but in part from distant centres—sometimes far distant. There is therefore no special advantage in considering the 486 localities separately. Instead, they will be grouped, in the first instance, into the eight " areas " recognised by the Ministry of Food and listed in Table 1.

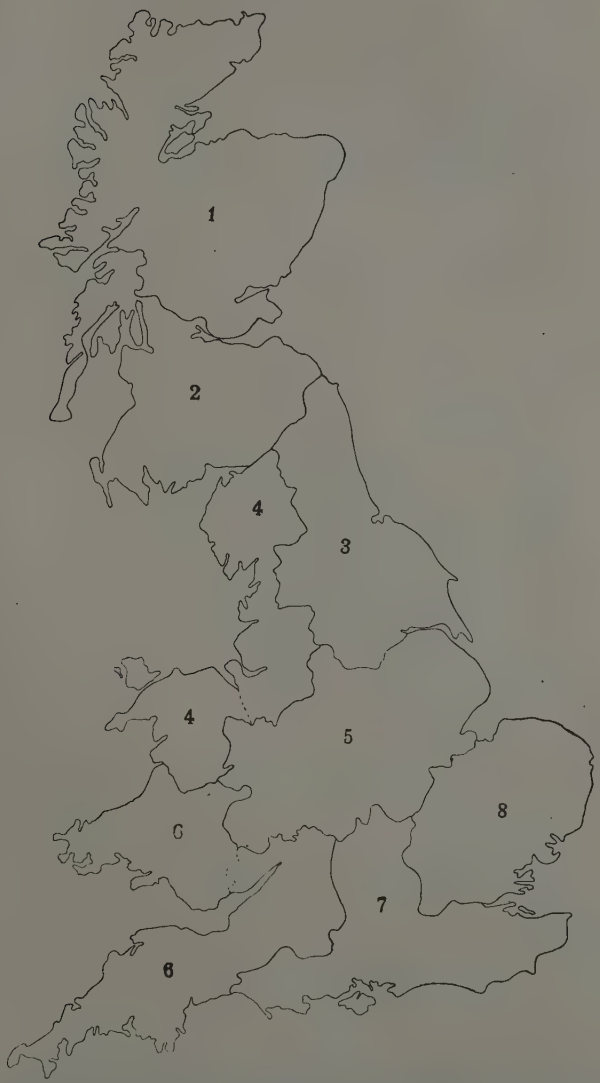
It will be seen that the cattle slaughtered are by no means proportioned equally amongst the eight areas. As against an expected percentage of 12·5 (on the basis of equal proportions), South England had 5% and South Scotland 22·2% of the total cattle. There is no special reason why the areas should be equal, but their marked inequality entails caution in drawing comparisons.

Class.

The classification into (i) Steers, heifers and cow heifers, (ii) Cows and (iii) Bulls was adopted because it is routine practice at the abattoirs : the regular weekly reports are drawn up in the same way. The actual totals of slaughtered cattle were as follows :—

Steers, etc. :	56,013 or 76·341% of the grand total ;	S.E. 0·1569%.
Cows :	14,464 or 19·713% of the grand total ;	S.E. 0·1469%.
Bulls :	2,895 or 3·946% of the grand total ;	S.E. 0·0719%.

As to time, these percentages are not constant over the three weeks. The proportion of steers (and their absolute numbers) is falling, whilst



Map showing the approximate boundaries of the eight areas. Dotted lines show the subdivision of areas 4 and 6 to form a new area: Wales.

that of both cows and bulls is increasing. As to area, the above percentages are widely departed from : Area 2 has the highest proportion of steers (88·9%) and Area 6 the lowest (52·9%) : vice versa, Area 6 has the highest proportion of cows (39·2%) and Area 2 the lowest (9·1%) : Area 7 has the highest proportion of bulls (8·4%) and Area 1 the lowest (1·6%). These departures from uniformity greatly complicate the process of drawing valid inferences from the actual infestation data.

TABLE 1.

Percentages of 73,372 Cattle and 486 Abattoirs by Areas.

Area No.	Counties Included	Cattle %	Abattoirs %
1	(**N. Scotland**) Aberdeen, Banff, Caithness, Forfar, Inverness, Kincardine, Moray, Nairn, Perth, Ross and Cromarty, Sutherland...	8·9	8·8
2	(**S. Scotland**) Argyll, Ayr, Berwick, Clackmannan, Dumbarton, Dumfries, Edinburgh, Haddington, Lanark, Linlithgow, Kirkcudbright, Peebles, Renfrew, Roxburgh, Selkirk, Stirling, Wigtown	22·2	12·3
3	(**N.E. England**) Durham, Northumberland, Yorks.	12·9	13·2
4	(**N.W. England, N. Wales**) Anglesey, Caernarvon, Cheshire, Cumberland, Denbigh, Flint, Lancs., Merioneth, Montgomery, Westmorland... ...	11·3	16·9
5	(**Central England**) Derby, Hereford, Leicester, Lincoln, Northants, Nottingham, Rutland, Salop, Staffs, Warwick	12·8	12·5
6	(**S.W. England, S. Wales**) Brecon, Cardigan, Carmarthen, Cornwall, Devon, Glamorgan, Gloster, Monmouth, Pembroke, Radnor, Somerset ...	7·6	16·3
7	(**S. England**) Berks, Dorset, Hants, Kent, Oxford, Surrey, Sussex	5·0	10·9
8	(**S.E. England**) Bedford, Buckingham, Cambridge, Essex, Hertford, Huntingdon, Middlesex, Norfolk, Suffolk	19·3	9·1
		100·0	100·0

Origin.

While the division into Home-bred and Imported cattle is likely to be a most informative one, the third group for cattle of unknown origin is merely an unfortunate necessity. The totals of slaughtered cattle falling under this classification were :—

Home-bred : 56,195 or 76·589% of the grand total ; S.E. 0·1563%.
Imported : 8,026 or 10·939% of the grand total ; S.E. 0·1152%.
Unknown : 9,151 or 12·472% of the grand total ; S.E. 0·1220%.

Actually, Glasgow, with 6,556 cattle all returned as unknowns is responsible for nearly 72% of the unknowns ; had all the Glasgow cattle been distributed between the other two groups the unknowns would have been 2,595 or only 3·537% of the grand total.

TABLE 2.

Percentages of 73,372 Cattle by Origin and Class.

	% of Origins by Classes							
Origin	Home-bred		Imported		Unknown		Total	
Class	%±S.E.		%±S.E.		%±S.E.		%±S.E.	
Steers	72·3	0·19	95·6	0·23	84·6	0·34	76·4	0·16
Cows	22·9	0·18	4·2	0·23	13·7	0·33	19·7	0·15
Bulls	4·8	0·09	0·2	0·05	1·7	0·14	3·9	0·07
Total ...	100·0		100·0		100·0		100·0	
N.	56,195		8,026		9,151		73,372	

	% of Classes by Origins							
Origin	Home-bred		Imported		Unknown		Total	N
Class	%±S.E.		%±S.E.		%±S.E.		%	
Steers ...	72·5	0·19	13·7	0·15	13·8	0·15	100·0	56,013
Cows ...	89·0	0·26	2·3	0·13	8·7	0·24	100·0	14,464
Bulls ...	94·0	0·44	0·7	0·16	5·3	0·41	100·0	2,895
Total	76·6	0·16	10·9	0·12	12·5	0·12	100·0	73,372

As to time, there is no change in relative proportions as between the first and second weeks, though the numbers from all " origins " are falling. The fall continues in the third week, but the proportions are changed : there is a 4% rise in the home-bred, and a corresponding fall in the unknowns. As to place, the greatest disturbance in the average proportions occurs in Area 2 where, as already described, the large numbers of cattle at Glasgow were all returned as " Unknown." The percentages for Area 2 were : Home-bred, 47·5 ; Imported, 10·0 ; Unknown, 42·5. Excluding Area 2, Home-breds formed the highest

proportion in Area 6 (96·4%) and lowest in Area 3 (77·1%); Imported were highest in Area 4 (18·7%) and lowest in Area 6 (1·1%); Unknown were highest in Area 7 (8·5%) and lowest in Areas 4 and 5 (both 2·0%). The highest proportion of imported cattle in Area 4 is due to the large-scale importation of Irish cattle at Birkenhead.

As to class, the relationships between Class and Origin are set out in Table 2; the upper half shows the percentages in which the various Origins are distributed among Classes, and the lower half shows the reverse, Classes distributed among Origins. The two classifications are not independent, there being an excess over expectation of cows and bulls among home-bred cattle and of steers, etc., among imported cattle. The left half of the table shows that the Unknown group falls between the Home-bred and Imported suggesting, as would be expected, that it is a mixture of both.

Degree of Infestation.

The preceding heads of classification have been plain matters of fact; the four-fold subdivision of Degree of Infestation on the other hand, involves a variable amount of luck and is largely a matter of judgment. If a liver is only lightly infested it is a question of chance whether or not the Inspector will see the flukes, and the probability of his doing so has a value quite unknown to the writers. If he sees them, it is a matter of judgment whether he decides on no condemnation, partial condemnation, or complete condemnation of the liver. This judgment may be expected to vary from one Inspector to another, but the combination into 8 areas consisting of between 43 and 82 abattoirs should effectively smooth out the personal variation. It is still possible that standards will vary from area to area, and this point cannot readily be decided, since in a more flukey area one would expect the proportion of total condemnations to be higher.

There is the further point that, while a cirrhotic ox liver is commonly ascribed to fluke, cirrhosis may be due to other causes, notably (according to information given the Committee by Mr. Cabot) the routine feeding to cattle of brewer's grains. An attempt was made to circumvent this difficulty by the wording of the form: " Liver *affected with fluke* causing partial (or total) condemnation "; there remains the possibility that, during a busy period, cirrhotic livers may have been counted as flukey without further evidence. On the other hand, where a carcase has been condemned for (say) tuberculosis, the liver might

also have contained fluke, which would not normally be seen and recorded. We know from the remarks on some forms that one or two Inspectors have gone to the trouble of examining (for fluke) livers condemned on other grounds, but this cannot be assumed to have been the general practice. It is fortunate that the two classes, non-distomatic cirrhotic livers counted as fluke, and distomatic livers condemned on other grounds and uncounted, must tend to cancel each other out.

The relationships between Degree of Infestation and the other main heads of classification constitute the next section of this report. It has been the purpose of this introductory section to indicate the simplifications which have been made (amalgamation of 486 abattoirs into 8 areas, and the use of three-weekly totals in place of data for separate weeks), and to discuss the problems raised by the fact that the main classificatory heads are not independent.

The Data on Fluke Infestation.

It is quite impracticable to list the data from each abattoir separately, and to give all the very numerous totals and sub-totals under each main head. Instead, the area totals, fully dissected under other heads, but without further summation, have been appended to the report in the form of Table 8. This gives the separate area totals for each Degree of Infestation under each Class and Origin, separately for each week. Nearly all the totals and percentages discussed in the report can be built up from this Table.

Infestation and Time.

This has already been discussed in the Introduction, where it was shown that there is a significant increase in the incidence of infestation in the third week. As the number of flukey cattle in the third week was less than in the preceding weeks, the increasing incidence is merely relative to the total number of cattle slaughtered; in other words, there were disproportionately fewer clean cattle in the third week.

Infestation and Place.

In Table 3, the third line under each area gives the percentages of all cattle livers falling under the four degrees of infestation: clean, flukey but not condemned, flukey and partially condemned, and flukey and wholly condemned. In each line the percentages add horizontally to 100 and the last column shows the total number on which they are based. Below the eight Areas come the totals for the country as

a whole, and here again the third line gives the percentages of all livers infested. Thus, over the whole country, 17·6% of cattle livers were found to be flukey, of which 4·7% were not condemned, 7·7% were partially, and 5·2% wholly condemned. It will be seen that the cleanest Areas are 7 and 8, south and south-east England, and the most flukey are 4 and 6, N.W. England with North Wales and S.W. England with South Wales; moreover, this major difference is broadly reflected in each of the degrees of infestation.

The total line for each area gives the percentage of all livers under each degree of infestation. If the clean livers are now omitted and the other three degrees are expressed as percentages of all *flukey* cattle, the values for the country as a whole under the degrees 1, 2 and 3 are 26·7%, 43·7% and 29·6% respectively. Thus, more flukey livers are classed as partially condemned than as either of the other groups. If the same is done for each Area separately the results are curiously anomalous and are not obviously related even to the incidence of infestation in those areas. This can be illustrated by the percentages of flukey livers which are totally condemned, given below :—

Area	1	2	3	4	5	6	7	8	*Total*
Degree 3 %	32·2	38·5	20·4	27·3	27·6	32·3	36·8	23·9	29·6
Infestation %... ...	17·7	14·8	16·7	28·6	16·7	25·7	13·1	13·8	17·6

(The third line is the percentage of *all* cattle found flukey). If now a typically flukey area, No. 4, is compared with a clean area, No. 7, it would appear that it is in the clean area that a higher proportion of flukey livers is totally condemned, suggesting that in a clean area the standards are more stringent. But now if the other most flukey area, No. 6, is compared with the other cleanest area, No. 8, the reverse is found: a higher proportion of flukey livers is totally condemned in the more flukey area. This may be merely a question of varying standards in different parts of the country, or it may be a matter of varying intensity of infestation; thus, it might be that the few flukey livers of Area 7 and the many of Area 6 show a greater degree of damage due to fluke than do those of Areas 8 and 4. Data are not available for deciding this question.

Infestation and Origin.

In Table 3, the first two lines for each Area and for the country as a whole show the percentages of home-bred and imported cattle, respectively, falling under each degree of infestation. The third origin, "Unknown," is omitted as being a mixture of the other two in

TABLE 3.

Percentage Infestation of Cattle by Area and Origin.

Area	Origin	Degree of Infestation								N
		0		1		2		3		
		%	±S.E.	%	±S.E.	%	±S.E.	%	±S.E.	
1. North Scotland	Home-bred	85·5	0·48	6·2	0·33	4·4	0·29	3·9	0·26	5,483
	Imported...	68·2	1·62	7·9	0·95	9·5	1·04	14·4	1·21	809
	Total ...	82·3	0·47	6·6	0·33	5·4	0·28	5·7	0·29	6,542
2. South Scotland	Home-bred	86·5	0·39	2·7	0·19	5·3	0·26	5·5	0·26	7,705
	Imported...	68·6	1·14	4·3	0·51	11·9	0·80	15·2	0·89	1,635
	Total ...	85·2	0·28	4·1	0·16	5·0	0·17	5·7	0·18	16,256
3. N.E. England	Home-bred	87·5	0·38	4·0	0·23	5·9	0·28	2·6	0·19	7,284
	Imported...	67·1	1·12	7·6	0·64	19·0	0·95	6·3	0·58	1,751
	Total ...	83·3	0·38	4·9	0·22	8·4	0·28	3·4	0·19	9,448
4. N.W. England	Home-bred	73·1	0·54	6·5	0·31	13·0	0·42	7·4	0·33	6,613
	Imported...	62·5	1·22	6·7	0·64	20·8	1·02	10·0	0·76	1,563
	Total ...	71·4	0·50	6·5	0·27	14·3	0·38	7·8	0·30	8,342
5. Central England	Home-bred	84·0	0·40	4·6	0·23	7·1	0·28	4·3	0·22	8,569
	Imported...	76·3	1·65	3·7	0·75	12·5	1·29	7·5	1·04	654
	Total ...	83·3	0·38	4·5	0·21	7·6	0·27	4·6	0·22	9,415
6. S.W. England	Home-bred	74·7	0·60	5·2	0·31	11·9	0·44	8·2	0·37	5,364
	Imported...	62·8	6·40	5·1	2·88	18·6	5·05	13·5	4·43	59
	Total ...	74·3	0·58	5·2	0·30	12·2	0·44	8·3	0·37	5,563
7. South England	Home-bred	86·5	0·61	2·4	0·27	6·3	0·43	4·8	0·38	3,215
	Imported...	76·3	3·62	3·0	1·48	12·6	2·84	8·1	2·36	135
	Total ...	86·7	0·56	2·4	0·26	6·0	0·40	4·9	0·36	3,661
8. S.E. England	Home-bred	89·3	0·28	3·6	0·17	4·5	0·19	2·6	0·15	11,962
	Imported...	61·9	1·29	6·5	0·66	21·7	1·09	9·8	0·80	1,420
	Total ...	86·2	0·29	4·1	0·17	6·4	0·21	3·3	0·15	14,145
Total ...	Home-bred	84·0	0·15	4·4	0·086	7·0	0·034	4·6	0·088	56,195
	Imported...	66·6	0·53	6·2	0·27	16·8	0·42	10·4	0·34	8,026
	Total ...	82·4	0·14	4·7	0·078	7·7	0·098	5·2	0·082	73,372

unknown proportions. Looking first at the totals for the whole country, it will be seen that the imported cattle are about twice as flukey as the home-bred. If now the percentages under degrees 1, 2 and 3 are expressed as percentage of *flukey* livers (instead of as percentage of *all* livers), the results are as follows :—

	Degree of Infestation	1	2	3
% of Flukey livers	Home-bred...	27·5	43·8	28·7
	Imported	18·6	50·3	31·1

This strongly suggests that the intensity, as well as the incidence, of infestation is higher among imported cattle.

A curious point is somewhat concealed by omitting the values for cattle of unknown origin : the incidence rate for these is lower than the other two groups, instead of falling between them as might be expected. The actual values for the whole country are as follows :—

Degree of Infestation	0	1	2	3	N
% Incidence, " Unknowns "	85·75	5·74	4·22	4·29	9,151

The reason for this appears to be that Glasgow, accounting for the majority of cattle of unknown origin, normally has access to cattle cleaner than the average : whether home-bred or imported is, of course, not known.

Turning now to the separate areas, the total percentage of flukey home-bred cattle (100—clean%) is highest in Area 4 (26·9%) and Area 6 (25·3%), and lowest in Area 8 (10·7%) and Area 3 (12·5%), with Areas 2 and 7 (13·5%) slightly above. The difference between the highest and lowest is $16{\cdot}2 \pm 0{\cdot}57\%$. The total percentage of flukey imported cattle is highest in Area 8 (38·1%) and lowest in Area 5 (23·7%), the difference being $14{\cdot}4 \pm 2{\cdot}25\%$.

Infestation and Class.

Table 4 is similar in construction to Table 3, but with Origins replaced by the three Classes : (i) Steers, heifers and cow heifers ; (ii) Cows ; and (iii) Bulls. The third-line totals of Table 3 are not repeated here. For the country as a whole it will be seen that cows have an incidence about twice as high as steers or bulls. The three degrees of infestation above zero, expressed as percentages of *flukey* livers, are as follows :—

Degree of Infestation	1	2	3
Steers, etc.	30·2	43·8	26·0
Cows	20·4	43·1	36·5
Bulls	28·1	44·5	27·4

TABLE 4.

Percentage Infestation of Cattle by Area and Class.

Area	Class	Degree of Infestation								N
		0		1		2		3		
		%	±S.E.	%	±S.E.	%	±S.E.	%	±S.E.	
1. North Scotland	Steers, etc.	82·9	0·52	6·9	0·35	5·2	0·31	5·0	0·30	5,420
	Cows ...	78·6	1·19	4·7	0·63	6·6	0·73	10·1	0·89	1,019
	Bulls ...	90·3	2·90	5·8	2·30	3·9	1·92	0·0	—	103
2. South Scotland	Steers, etc.	86·4	0·29	4·0	0·16	4·6	0·17	5·0	0·18	14,458
	Cows ...	74·2	1·13	5·2	0·58	8·4	0·73	12·2	0·86	1,478
	Bulls ...	83·1	2·08	5·0	1·21	6·9	1·41	5·0	1·21	320
3. N.E. England	Steers, etc.	83·8	0·43	5·0	0·25	8·2	0·32	3·0	0·20	7,542
	Cows ...	79·7	1·02	4·8	0·54	9·9	0·77	5·6	0·58	1,549
	Bulls ...	88·9	1·64	3·6	0·99	5·3	1·19	2·2	0·79	357
4. N.W. England	Steers, etc.	80·3	0·59	4·4	0·31	11·0	0·46	4·3	0·30	4,528
	Cows ...	57·6	0·88	9·4	0·52	19·2	0·71	13·8	0·62	3,205
	Bulls ...	78·4	1·67	6·4	1·00	12·6	1·33	2·6	0·65	609
5. Central England	Steers, etc.	86·9	0·42	3·5	0·23	6·8	0·31	2·8	0·21	6,527
	Cows ...	73·2	0·91	7·3	0·53	10·7	0·63	8·8	0·58	2,397
	Bulls ...	84·3	1·62	4·5	0·94	3·9	0·88	7·3	1·17	491
6. S.W. England	Steers, etc.	84·4	0·67	4·6	0·39	7·8	0·50	3·2	0·33	2,941
	Cows ...	59·6	1·05	6·0	0·52	18·5	0·84	15·9	0·82	2,184
	Bulls ...	79·8	1·92	5·0	1·05	10·0	1·42	5·2	1·06	438
7. South England	Steers, etc.	90·3	0·65	2·2	0·33	4·5	0·46	3·0	0·38	2,073
	Cows ...	79·4	1·13	3·1	0·49	9·4	0·83	8·1	0·78	1,282
	Bulls ...	92·5	1·50	0·3	0·38	2·6	0·92	4·6	1·19	306
8. S.E. England	Steers, etc.	86·7	0·31	4·2	0·18	6·2	0·22	2·9	0·15	12,524
	Cows ...	80·5	1·08	3·3	0·49	9·2	0·80	7·0	0·71	1,350
	Bulls ...	93·4	1·50	1·8	0·82	1·5	0·75	3·3	0·09	271
Total ...	Steers, etc.	85·4	0·15	4·4	0·087	6·4	0·10	3·8	0·080	56,013
	Cows ...	70·1	0·38	6·1	0·20	12·9	0·28	10·9	0·26	14,464
	Bulls ...	84·7	0·68	4·3	0·38	6·8	0·47	4·2	0·38	2,895

This shows that the intensity of infestation also is higher in cows than in steers or bulls. The reason why cows are more affected than steers, etc., is probably (*a*) that a longer life increases the exposure to infestation, combined with the fact (*b*) that an infestation once set up will probably last for many years. This latter fact is frequently denied by animal husbandry experts, possibly because the acute stage of the disease is usually short-lived, but there is good evidence that flukes can and do live as long as their hosts. That this argument does not apply to bulls is probably because they (*a*) are generally better cared for and (*b*) spend more of their time in stalls, where they are less exposed to infestation.

On examining the separate areas it will be found that Areas 4 and 6, covering the west of the country, are abnormally flukey largely on account of (*a*) the high incidence of fluke in cows in these two areas, and (*b*) the high proportion of cows to other classes in these two areas. Thus, respectively 42·4% and 40·4% of cows were infested with fluke as compared with 29·9% of cows for the whole country; and respectively 38·5% and 39·3% of all slaughtered cattle were cows as compared with 19·7% of all cattle for the whole country. In these same two areas the incidence of fluke in bulls is higher than elsewhere. As for steers, while Area 4 is the worst by a small margin, Area 6 is rather better than Areas 1 and 3.

Further Analysis in the West.

Tables 3 and 4 suggested that Areas 4 and 6 were particularly bad in relation to home-bred cows. Moreover, from the point of view of fluke endemic in this country, it is the data for home-bred cattle which are informative. Between them, Areas 4 and 6 cover the whole of Wales and Western England, and it was felt desirable to make a third area in this region by separating the North Wales data from Area 4 and the South Wales from Area 6, combining North and South Wales, and so making three western areas roughly comparable in size and in numbers of cattle recorded. This has been done in respect of home-bred cattle only, in Table 5, which gives the degree of infestation by classes for the three new areas, for the total of the three, and for the rest of the country (i.e. excluding the three). This table shows that all classes of home-bred cattle in the west are more heavily infested than cattle elsewhere; indeed the proportions of flukey cows and bulls are almost double those found in the rest of the country. If now the

TABLE 5.

Western Home-Bred Cattle by Area and Class.

Area	Class	Degree of Infestation								N
		0		1		2		3		
		%	±S.E.	%	±S.E.	%	±S.E.	%	±S.E.	
N.W. England	Steers ...	86·8	0·64	4·0	0·37	7·2	0·49	2·0	0·27	2,798
	Cows ...	58·5	0·94	9·0	0·54	19·4	0·76	13·1	0·65	2,773
	Bulls ...	73·6	1·90	6·7	1·08	13·3	1·47	2·4	0·64	540
Wales ...	Steers ...	79·3	1·32	5·0	0·73	12·3	1·09	3·4	0·61	920
	Cows ...	45·4	1·78	8·9	1·02	24·8	1·53	20·9	1·43	795
	Bulls ...	81·3	2·63	5·6	1·58	8·9	1·94	4·2	1·37	213
S.W. England	Steers ...	87·2	0·71	4·1	0·42	5·7	0·49	3·0	0·36	2,249
	Cows ...	65·8	1·26	5·6	0·62	14·2	0·93	14·4	0·94	1,426
	Bulls ...	79·8	2·48	3·8	1·18	10·3	1·88	6·1	1·48	263
Total of Above	Steers ...	85·8	0·45	4·2	0·26	7·4	0·34	2·6	0·21	5,967
	Cows ...	58·5	0·70	8·0	0·38	18·8	0·55	14·7	0·50	4,994
	Bulls ...	79·0	1·26	5·7	0·74	11·6	1·00	3·7	0·60	1,012
Remainder (Total — 4+6)	Steers	88·9	0·17	3·8	0·10	4·6	0·11	2·7	0·087	34,637
	Cows ...	77·5	0·47	4·6	0·24	9·7	0·33	8·2	0·31	7,876
	Bulls ...	88·3	0·78	3·0	0·42	4·2	0·49	4·5	0·51	1,705

totally condemned livers are expressed as a percentage of all flukey livers in each class, the results are somewhat surprising :

Class	*Steers, etc.*	*Cows*	*Bulls*
West Region	18·3±1·33%	35·4±1·02%	17·6±2·52%
Elsewhere	24·3±0·69%	36·5±1·13%	38·5±3·45%

Thus, a lower percentage of flukey livers from steers and bulls is totally condemned in the west of the country than in the remainder : the difference in respect of cows is not significant. Moreover, while in the west more cow livers are totally condemned than those of other classes, as might be expected from the fact that the incidence of fluke is much higher in cows, in the rest of the country as high a proportion of bull livers as of cow livers is totally condemned ; yet the incidence in cows is about double that in bulls in the same region. It is difficult to avoid the conclusion that in the west bulls are less intensely infested than cows, while elsewhere the intensity of infestation is about the same.

TABLE 6.
Economic Loss to Meat Industry.

Class or Origin	No. of Condemned Livers	Annual Rate of Loss			Loss as percentage of			
		Weight tons	Wholesale £	Retail £	All Cattle		Total Loss	
					%	±S.E.	%	±S.E.
Steers, etc.	3925·5	397·694	38,982	49,614	7·01	0·11	59·0	0·60
Cows ...	2503	242·112	24,856	31,635	17·3	0·31	37·7	0·59
Bulls ...	220·5	21·328	2,190	2,787	7·62	1·79	3·3	0·22
Total ...	6649	643·134	66,028	84,036	9·06	0·106	100	
Home-bred	4549·5	440·050	45,179	57,501	8·10	0·12	68·4	0·57
Imported	1513·5	146·399	15,030	19,129	18·9	0·44	22·8	0·51
Unknown	586	56·685	5,820	7,406	6·41	0·26	8·8	0·35

Turning now to the three new areas, evolved from Areas 4 and 6, it will be seen that steers and cows show a higher incidence of fluke in Wales than in N.W. or in S.W. England. Curiously, this does not apply to bulls ; indeed, the difference between Wales and N.W. England, in favour of Wales, is probably significant (7·7 ± 3·25%). Wales has the distinction of being the only area where more than 50% of cows harboured fluke ; it is in this respect significantly worse than in the north-west, which in turn is significantly worse than the south-west ; but in the whole of the west home-bred cows are very heavily infested. One may say that, as far as home-bred cattle are concerned, fluke centres pre-eminently in cows as to class, and in Wales as to area.

It was hoped to locate the Welsh flukey areas with more precision by extracting from the forms, from Welsh abattoirs, two lists of collecting centres, viz., collecting centres recorded by those abattoirs which showed a fluke incidence of, respectively, more and less than 50%. This did not prove very fruitful, partly because the same collecting centre frequently appeared on both lists, and partly because, of the total of 99 collecting centres, 26 were outside Wales and Monmouth. It is therefore considered that further information on the most flukey areas is to be obtained not by further analysis of these forms, but by a fresh enquiry based on selected, flukey abattoirs.

Economic Loss.

Table 6 represents an attempt to assess the economic loss to the meat industry caused by fluke in cattle, and is based on condemned livers only. The first column gives the subdivisions of the two categories "Class" and "Origin" with the total, common to both categories. The second column gives the actual numbers of livers condemned during the three weeks, on the simplifying assumption that exactly half of the partially condemned livers are available for sale: this explains the fractions of column 2. The next three columns give the loss, *at the annual rate,* in three different units: (*a*) Column 3 gives the weight in tons per annum on the assumption that the average weight of an ox liver is 12·5 lb.; (*b*) Column 4 gives the wholesale value of the condemned livers in pounds sterling per annum at the assumed price of 11d. per lb.; (*c*) Column 5 similarly gives the retail value at 1s. 2d. per lb. Column 6 expresses the loss under each subdivision as a percentage of the total livers available from all slaughtered cattle in that subdivision. Finally, the last column expresses the loss under each subdivision as a percentage of the total loss.

The incidence rate based on all degrees of infestation above zero is almost exactly twice as high among cows as among steers, etc. (1 to 2·05), and similarly among imported cattle as among home-bred (1 to 2·09). If instead the rate is based only on condemned livers plus half the partially condemned, then the proportions are still higher, as Column 6 of the table shows, where the ratio of steers to cows is as 1 to 2·47, and that of home-bred to imported is as to 1 to 2·34. This might suggest that the economic loss was greatest in the subdivisions "cows" and "imported." But, because the actual number of steers is greater

than that of cows in the ratio 3·88 to 1, the economic loss is in fact greater in steers than in cows, in the ratio 1·57 to 1, as is shown in Column 7. Similarly, because home-bred cattle exceed imported in the ratio 7.03 to 1, the loss is greater among the former in the ratio 3·01 to 1. Again, although the condemnation rate among bulls does not differ significantly from that of steers, bulls are so few that they account for only some 3% of the total loss.

From the lack of homogeneity in the data of this survey and the presence of interactions between categories, it is obvious that too much reliance must not be placed on Table 6. In particular, while the losses over the three weeeks were certainly somewhere near the rates shown, the losses over a year or more might well be entirely different. There is good circumstantial evidence, outside the limits of this survey, that the true annual losses are very much higher. This evidence is disclosed in the next Section. Meanwhile, the losses of Table 6 should probably be regarded as minimum values.

Additional Data.

In Table 7 certain additional and earlier data from the abattoirs at Birmingham, Birkenhead, Liverpool, Glasgow and Edinburgh, are compared with the data from each abattoir in the present survey. Direct comparison is not always easy because the precise wording of the questionnaire used in the survey cannot always be applied to the earlier data. The following notes will explain what is meant.

(*a*) *Birmingham's* additional data relate to the whole year 1941, the number in the " Cattle Affected " column being the livers affected with fluke and condemned (presumably, totally condemned) on that ground. It is pointed out that 4,293 livers condemned for tuberculosis and 1,413 for other reasons may have included some flukey livers, which would not be counted as such. The survey data appropriate to the comparison would therefore be those of the third degree, " Affected with fluke causing total condemnation." These are given in the next line and show a significantly lower incidence rate.

(*b*) Both *Birkenhead* and *Liverpool* send data for the three years from 1939 to 1941, and it is specifically stated that " Only those animals are taken as affected in which the whole of the liver . . . has been seized for fluke and condemned totally." The data are therefore strictly comparable with our third degree. In both cases the 1942

survey shows a lower incidence rate than in the previous three years. All Birkenhead cattle are Irish, whilst those at Liverpool are mixed.

(*c*) From *Glasgow* come data not merely sorted into home-bred and imported cattle, but subdivided under home-bred into 18 Scottish collecting centres. These have been combined in Table 7 to give a

TABLE 7.

Additional Data.

Centre	Data	Numbers of Cattle		Affected %	
		Slaughtered	Affected	%	±S.E.
Birmingham	1941	56,690	4,574	8·07	0·12
	Survey	1,572	93	5·92	0·60
Birkenhead	1939	38,585	8,063	20·90	0·21
	1940	76,443	17,961	23·50	0·15
	1941	136,583	19,892	14·56	0·10
	Survey	558	57	10·22	1·28
Liverpool	1939	74,293	13,951	18·78	0·14
	1940	37,594	16,755	44·57	0·25
	1941	34,969	11,798	33·74	0·25
	Survey	912	52	5·70	0·77
Glasgow	1941	35,415	13,222	37·33	0·25
	Survey	6,556	690	10·52	0·38
Edinburgh	1925	31,568	5,147	16·30	0·21
	1926	31,652	7,044	22·25	0·23
	1927	33,000	8,628	26·15	0·24
	1928	32,426	8,574	26·44	0·24
	1929	33,044	8,893	26·91	0·24
	1930	33,207	9,720	29·27	0·25
	1931	31,250	7,740	24·77	0·24
	1932	30,697	8,462	27·57	0·25
	1933	32,551	8,770	26·94	0·24
	1934	33,541	6,489	19·35	0·22
	1935	35,384	4,405	12·45	0·18
	1936	38,452	3,919	10·19	0·16
	1937	35,824	5,846	16·32	0·19
	1938	34,175	7,245	21·20	0·22
	1939	35,007	7,294	20·84	0·22
	1940	27,440	5,906	21·52	0·25
	1941	22,638	3,797	16·77	0·25
	Total	551,856	117,879	21·36	0·06
	Survey	2,345	141	6·01	0·49
			(169)	(7·21)	(0·54)
Totals	Additional	1,042,428	224,095	21·50	0·04
	Survey	11,943	1,033	8·65	0·26

TABLE 8.

Area Totals fully Dissected.

Area	Class	Infestation											
		0 Uninfested			1 Uncondemned			2 Part Condemned			3 Fully Condemned		
		H–B	I	U	H–B	I	U	H–B	I	U	H–B	I	U
1	Steers, etc.	1,179	125	29	112	24	8	52	28	4	43	28	17
		1,333	174	34	86	21	4	70	27	6	58	41	10
		1,379	209	30	100	13	8	65	17	14	34	24	14
	Cows ...	238	10	20	16	4	3	22	3	3	25	10	1
		214	17	20	10	2	0	14	1	2	27	12	1
		252	16	14	12	0	1	17	1	4	25	2	0
	Bulls ...	27	0	1	1	0	0	1	0	0	0	0	0
		35	0	0	1	0	0	1	0	0	0	0	0
		28	0	2	4	0	0	2	0	0	0	0	0
2	Steers, etc.	2,359	273	2,378	54	19	96	98	52	74	106	57	75
		2,172	481	2,038	62	17	119	90	72	77	107	113	60
		1,378	368	1,038	60	34	113	97	71	40	84	75	51
	Cows ...	190	0	210	8	0	26	36	0	10	39	0	19
		181	0	208	6	0	12	31	0	3	41	2	11
		192	0	116	12	0	13	36	0	8	31	0	37
	Bulls ...	73	0	16	4	0	4	5	0	0	3	1	2
		48	0	34	1	0	1	9	0	1	5	0	0
		72	0	23	4	0	2	7	0	0	4	0	1
3	Steers, etc.	2,445	610	115	115	76	11	121	190	4	49	60	5
		1,550	401	69	63	48	9	116	110	1	36	40	3
		968	142	24	45	6	0	46	30	2	22	10	0
	Cows ...	342	16	62	27	1	4	39	0	7	25	1	4
		370	0	29	15	1	1	31	3	3	25	0	7
		392	3	21	21	1	3	65	1	4	24	0	1
	Bulls ...	104	1	5	0	0	5	4	0	4	4	0	0
		100	0	5	6	0	0	5	0	0	3	0	0
		97	0	5	2	0	0	6	0	0	1	0	0
4	Steers, etc.	1,726	404	5	61	42	0	124	105	5	29	42	2
		610	75	24	36	20	0	56	25	0	25	23	0
		357	433	1	24	16	1	46	138	0	12	61	0
	Cows ...	543	24	35	106	10	1	174	19	2	128	13	1
		522	19	48	77	7	3	189	17	0	121	6	3
		613	17	25	89	7	0	196	20	0	157	11	2
	Bulls ...	152	4	4	16	1	0	22	1	0	4	0	1
		158	0	2	10	1	0	27	0	0	6	0	0
		155	1	1	10	1	0	27	0	0	5	0	0

NOTE.—H–B=Home-bred; I=Imported; U=Unknown. Each cell contains the three separate weekly totals.

TABLE 8—*concluded.*

Area	Class	Infestation											
		0 Uninfested			1 Uncondemned			2 Part Condemned			3 Fully Condemned		
		H–B	I	U	H–B	I	U	H–B	I	U	H–B	I	U
5	Steers, etc.	2,025	219	19	85	11	1	142	19	3	52	19	0
		1,653	172	47	68	5	0	96	35	4	39	18	1
		1,415	93	24	55	6	0	114	25	5	48	9	0
	Cows ...	534	1	19	50	1	6	80	1	7	57	2	8
		550	8	13	45	0	0	81	1	5	47	1	1
		612	5	11	66	1	6	77	1	4	90	0	6
	Bulls ...	142	1	0	7	0	0	3	0	0	11	0	0
		133	0	1	7	0	0	8	0	0	13	0	0
		136	0	1	8	0	0	8	0	0	12	0	0
6	Steers, etc.	765	2	5	33	3	0	62	4	1	17	4	0
		678	8	7	37	0	1	54	0	1	26	0	0
		981	21	15	60	0	2	102	5	1	46	0	0
	Cows ...	385	0	18	39	0	0	128	0	8	107	2	12
		367	2	14	44	0	1	93	1	3	94	0	1
		491	4	21	45	0	2	158	1	12	128	2	1
	Bulls ...	122	0	2	7	0	0	23	0	1	8	0	0
		106	0	7	6	0	0	11	0	0	6	0	0
		109	0	3	9	0	0	8	0	1	9	0	0
7	Steers, etc.	470	30	122	17	2	3	24	0	0	13	2	7
		564	48	77	11	2	0	25	13	0	16	3	0
		483	25	54	10	0	0	29	2	0	14	6	1
	Cows ...	296	0	8	10	0	0	36	0	1	33	0	1
		357	0	10	17	0	0	35	2	0	40	0	0
		337	0	10	11	0	2	46	0	0	26	0	4
	Bulls ...	88	0	5	1	0	0	1	0	0	1	0	0
		87	0	3	0	0	0	4	0	0	4	0	0
		98	0	2	0	0	0	3	0	0	8	0	1
8	Steers, etc.	3,147	359	231	146	37	10	129	99	3	55	50	3
		3,156	255	202	153	41	25	160	95	32	76	48	15
		3,097	244	169	89	13	15	132	107	15	75	40	1
	Cows ...	366	2	10	16	0	2	38	3	0	33	0	0
		335	7	9	7	1	0	32	2	0	38	0	1
		343	6	9	16	1	1	46	2	1	22	0	1
	Bulls ...	80	0	1	1	0	0	3	0	0	2	1	0
		73	1	3	3	0	0	1	0	0	4	0	0
		85	6	4	1	0	0	0	0	0	2	0	0

NOTE.—H–B=Home-bred; I=Imported; U=Unknown. Each cell contains the three separate weekly totals.

total comparable with the survey data, all of which were of unknown origin. "Cattle Affected" in this case means the number of livers affected with cirrhosis, and the point has already been made that cirrhosis is not necessarily due to fluke, but may be caused, for example, by feeding brewers' grains. In the additional data the number of affected cattle is therefore probably inflated. Having no subdivision in the survey comparable with cirrhosis, we have included all three degrees of infestation. Even so, there is a very marked drop in June, 1942, compared with the 1941 data, which relate to the quarter October-December.

(*d*) The additional *Edinburgh* data, going back to 1925, show the "Numbers of livers in which pathological change, as a result of fluke infestation, was sufficiently advanced to justify seizure." This presumably means total condemnation, and these are the data selected from the survey for comparison. But, in case the phrase includes partially condemned livers, these have been added to the data shown in parentheses. In either case the incidence rate for 1942 is very much lower than any preceding annual rate.

We are thus faced with the fact that at five major abattoirs the rate of condemnation revealed by this survey is abnormally low. This makes it highly probable that the average rate for the whole country is also below normal. The reason for this situation is not obvious, and can probably not be found from the data available. It is not even clear whether the whole year 1942 was abnormally free from flukey cattle, whether we chanced upon an unusually low three-weekly period, or whether there is a regular seasonal decline at this time of year (June). Whatever its duration, such a decline might be due to a sub-normal proportion of cows, or of imported cattle, or even to a less stringent standard of condemnation in war time, or to a combination of these factors. An abrupt fall is not likely to be due to a real reduction in the number of flukes in the country; the high relative incidence in cows suggests that the infestation persists many years, and this in turn suggests that any real reduction in flukes must lead to a *gradual* fall in incidence. The totals of Table 7 enable us to estimate roughly that the condemnations in a normal year are likely to be about 2·5 times as great as those indicated in the survey; this is probably something like the appropriate factor for increasing the loss-estimates of Table 6.

Summary and Conclusions.

1. An analysis of slaughter-house returns, covering over 73,000 cattle slaughtered during three weeks in June, 1942, reveals evidence of heterogeneity and interaction in respect of all the five categories involved: time, area, class, origin and degree of infestation with liver fluke. By heterogeneity is here meant that the differences between the various sub-divisions of any one category are too large to be ascribed to chance; by interaction is meant that the relative proportions between the subdivisions under any one category do not remain steady (within the limits of random variation) over the co-ordinate subdivisions of a second category. This makes the data difficult to interpret clearly.

2. The average incidence of fluke in cattle, based on the whole body of data, was $17{\cdot}668 \pm 0{\cdot}141$ per cent.

3. The frequency of fluke infestation in respect of the other four categories was as follows:—

(*a*) As to time, lowest: 1st week (16·7%); highest: 3rd week (19·3%).

(*b*) As to area, lowest: S. England (13·3%); highest: N.W. England with N. Wales (28·6%).

(*c*) As to origin, lowest: Unknown (14·2%); highest: Imported (33·4%).

(*d*) As to class, lowest: Steers, etc. (14·6%); highest: Cows (29·9%).

These relations apply to the data as a whole; owing to interaction, they cannot be assumed to apply to any subdivision.

4. Among degrees of infestation, omitting Degree 0 (uninfested), frequency was highest in the "partially condemned" subdivision on the average.

5. If the proportion of flukey livers which are totally condemned can be taken as an index of intensity of infestation, there is no obvious relationship between intensity and incidence in the various areas; but cows (as to class) and imported cattle (as to origin) show both the highest intensity and the highest incidence in their categories.

6. Further local analysis of home-bred cattle shows that Wales has the most flukey cattle, particularly cows, which happen to form an unduly large proportion of the total cattle slaughtered in that area.

Nevertheless, the proportion of flukey cow livers which are totally condemned is no higher in the west than elsewhere; and the proportion of flukey bull and steer livers which are totally condemned is significantly lower in the west than elsewhere. This implies either a lower standard of condemnation in the west or a lower intensity of infestation.

7. Economic loss to the meat industry was at the rate of over 600 tons of liver per annum, representing a retail price rate of nearly £100,000 per annum. More than half of this loss occurred among steers, etc., as to class, and among home-bred cattle as to origin.

8. Additional data from five large abattoirs, covering periods of time varying up to 17 years prior to 1942, agree in showing a much higher incidence of condemned livers than is revealed by the survey: on the average, 21·5% as against 8·6%. Reasons for this anomaly are not known, but it probably follows that the economic loss is normally about two and a-half times the estimate of the last paragraph.

9. Thus the normal loss in terms of retail price would be in the region of £200,000 per annum. But, in assessing the total loss due to fluke in cattle, it would be necessary to include reduced meat-, milk-, and possibly calf-production in infested animals; these factors are ill-defined but certainly large.

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The Metabolism of Trichinosed Rats during the Intermediate Phases of the Disease.

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Rogers (1941) has described the metabolic disturbances in rats caused by *Trichinella spiralis* up to 24 days after infection. The present work confirms the results obtained during the early phase of the disease and carries the investigation on a further 24 days. In general, the line of the previous work has been followed but it has been extended in that P and Ca metabolism have received some attention. The description of the urinary changes caused by infection is not included in this paper. The investigation has involved three separate groups of experiments each using two rats, the degree of intestinal and muscular infection varying from group to group (see Table 1).

Experimental Procedure.

As before, each experiment was divided into a series of four day periods, the faeces and urine of each period being collected separately. Details of the techniques were given previously (Rogers, 1941). Variations from the original procedure are given below.

a. Helminthological Procedure.

The experimental rats were kept in the metabolism cages and fed on the standard diet for at least 12 days before the collection of period (4 day) samples of urine and faeces was commenced. During this time the faeces was examined for helminths and eggs. Rats 7, 8, 9 and 10 were found to be uninfected and, after the commencement of the experiment proper, no further helminthological examination of the faeces was carried out. In the case of rats 5 and 6, however, very light *Oxyuris* infestations were found (it is estimated that the greatest number of eggs passed in a day was only 12) and hence samples of faeces were taken each period throughout the experiment and egg counts carried out. No consistent changes in the egg output were noted and it is considered that the very light *Oxyurius* infections present had no material effects on the course of the investigations.

After several periods during which the level of protein digestion, urinary N output, etc., of the uninfected rats was determined, the animals were fed with their food weighed amounts of fresh rat

diaphragm containing known numbers of *T. spiralis* larvae. At the end of the experiment the rats were killed and the diaphragms examined to estimate roughly the degree of infection (light, moderate or heavy). The fur was removed from the cadavers which were then minced, digested with artificial gastric juice and the total number of larvae in the digests estimated as before. Table 1 gives the number of larvae fed and the numbers found on post-mortem examination.

TABLE 1.

Showing the infective doses, the resulting degree of infestation and the general severity of the disease as indicated by disordered digestion and changes in the nature of the urine.

No. of animal.	No. of larvae fed.	Wt. (wet) of infective material.	Size of infection.		Reactions of rats to infection.
			Larvae in digest.	Larvae in diaphragm.	
5	830	0·40 g.	68,000	Moderate	Digestive changes—moderate Urinary changes—moderate
6	830	0·30 g.	37,000	Moderate	Digestive changes—moderate Urinary changes—moderate
7	2,100	0·80 g.	19,300	—	Digestive changes—marked Urinary changes—marked
8	2,100	0·95 g.	71,100	Heavy	Digestive changes—marked Urinary changes—marked
9	1,200	0·25 g.	1,400	Light	Digestive changes—moderate Urinary changes—slight
10	1,100	0·24 g.	750	Very light	Digestive changes—moderate Urinary changes—slight

In the case of rats 9 and 10 it would appear that infections were not established for very few larvae were found when counts were made on the digests or when the diaphragms were examined (this latter procedure was carried out as a rough check on the digestion technique). Further, the urines, though indicating the presence of worms during the early part of the experiment, soon became normal except for slight increases in volume, urea and *p*H. However, digestive disturbances were marked, protein assimilation being retarded and anorexia and diarrhoea occurring. This indicates that an intestinal infection was secured for it is unlikely that the fesh rat diphragm fed (at most, 0·25 g.wt.) could cause a disorder lasting at least 10 days. It is considered, therefore, that though adult worms became established in the intestines, few larvae were passed into the host, or if they were, they were soon destroyed and resorbed. A number of larvae, blackened and misshapen, were noted in the diaphragms. It may be possible that the diet, which was good in quality and plentiful in quantity, prevented the establishment of muscular parasites. The property of causing resorption of

T. spiralis larvae in tissue has been attributed to some diets by Motomura and Umeza (1936). It is doubtful if immunity could have given rise to the observed results for Culbertson (1942, 1942a) found that the immunity of rats to *T. spiralis* was directed largely towards the intestinal forms.

b. Diet.

Rats 5 and 6 were fed on a diet consisting of whole meal wheaten flour, dried " full cream " milk and NaCl fed twice daily in 6 g. lots as a paste mixed in 10 ml. of tap water plus 0·04 g. of cod liver oil. The diet of the remaining animals contained, in addition to the above constituents, ground maize, crushed linseed meal, " milk substitute powder," crude casein and calcium lactate. Rats 9 and 10 received 7 g. in 12·5 ml. of water containing 0·09 g. of cod liver oil twice daily. The remaining animals were given 6g. in 10 ml. of water plus 0·045 g. cod liver oil twice daily and were also given water ad lib. In every case, any food uneaten after about 9 hours was removed, dried, weighed and the amounts subtracted from the total period ration.

The water and protein content of the food was determined on 2 samples about every 12 days. As no consistent variations were noted in the food of rats 7, 8, 9 and 10, the results were averaged when calculating the period rations of each group. Two lots of food were used for rats 5 and 6 and though that fed during periods 12 to 15 was made up of materials similar to those used for the early part of the experiment, it showed, on analysis, a consistent slightly lower protein content. The figures for this second lot of food were therefore averaged separately. As the food was well mixed before presentation, no analyses were carried out on the uneaten food. Table 2 gives the results obtained in the analysis of food.

TABLE 2.

Showing the results of the food analysis. The figures within brackets are included to indicate the amounts of maximum variation from the average. Samples were weighed, oil and water was added and the lot mixed in the same way as each normal meal was prepared.

Rat No.	Average dry wt. of food fed each meal.	Average amount of protein fed each meal.
5, 6. Periods 1–12 ...	5·250 (±0·039) g.	0·701 (±0·027) g.
Periods 12–15 ...	5·208 (±0·072) g.	0·679 (±0·036) g.
7, 8	5·548 (±0·040) g.	1·371 (±0·015) g.
9, 10	6·229 (±0·029) g.	1·450 (±0·015) g.

In addition to the normal ration, small amounts of rat flesh were fed when the experimental animals were infected (for amounts see Table 1). No allowance for this has been made in the calculation of protein and N balances for rats 5, 6, 9 and 10 and hence the protein or N intake shown in Tables 3, 5, 7 and 9 are slightly lower than the true figures. Since the amounts of tissue fed to rats 7 and 8 was comparatively large, corrections, on the basis that the muscle contained 20 per cent. protein, have been made in Tables 4 and 7.

Protein Digestion.

Protein estimations were carried out by the Kjeldahl method using

Table 3.

Showing the amounts of protein digested by rats 5 and 6 before and after infection. The rats were infected at the beginning of period 5 as indicated by the dark line.

Period	Rat 5			Rat 6		
	Intake grams.	Voided grams.	Percentage digested.	Intake grams.	Voided grams.	Percentage digested.
1	5·608	1·193	78·73	5·604	1·108	80·22
2	5·608	1·120	80·06	5·604	1·155	79·38
3	5·608	0·910	83·78	5·604	0·915	83·60
4	5·608	1·250	77·78	5·604	0·955	82·95
5	5·608	1·362	75·73	5·604	1·680	76·02
6	5·608	1·538	72·60	5·604	1·320	70·45
7	5·608	1·158	79·33	5·604	0·957	82·93
8	5·608	1·100	80·40	5·604	0·991	82·34
9	5·608	0·949	83·10	5·604	0·989	82·36
10	5·608	0·918	83·65	5·604	0·841	85·00
11	5·608	1·001	82·15	5·604	0·716	87·22
12	5·433	1·074	80·29	5·433	0·771	85·85
13	5·433	0·988	82·00	5·433	0·770	85·85
14	5·433	0·949	82·51	5·433	0·748	86·28
15	5·433	0·855	84·38	5·433	0·831	84·71

a macro digestion and micro distillation. N × 6·2 was considered to give the amount of protein present. Figures, giving the averages obtained for each period, are shown in Tables 3, 4 and 5. " Intake " refers to the amounts of protein eaten, " voided," that passed in the faeces. Rats 5, 6, 9 and 10 were infected at the beginning of the 5th period while the remainder were infected at the beginning of the 4th.

TABLE 4.

Showing the amounts of protein digested by rats 7 and 8 before and after infection. The rats were infected at the beginning of period 4 as indicated by the dark line.

Period	Rat 7			Rat 8		
	Intake grams.	Voided grams.	Percentage digested.	Intake grams.	Voided grams.	Percentage digested.
1	10·970	1·781	83·77	10·970	1·803	83·57
2	10·970	1·810	83·51	10·970	1·880	82·87
3	10·970	1·766	83·91	10·970	1·840	83·23
4	11·130	2·310	79·25	11·160	2·080	81·37
5	8·987	2·858	68·22	10·137	2·869	71·70
6	9·317	2·663	72·51	9·601	2·860	70·22
7	10·970	1·926	82·46	10·970	2·120	80·86
8	10·970	1·749	84·06	10·970	1·851	83·13
9	10·970	1·860	83·05	10·970	1·860	83·05
10	10·970	1·980	81·96	10·970	1·992	81·85
11	10·970	1·778	83·80	10·970	1·861	83·05
12	10·970	1·937	82·00	10·970	1·821	83·60
13	10·970	1·794	83·00	10·970	1·810	83·50
14	10·970	1·912	82·67	10·970	1·860	83·04
15	10·970	1·962	82·09	10·970	1·901	82·68

As allowance for the addition of flesh to the normal ration of the first periods of infection was seldom made, the percentages of protein digested shown in the tables are lower than the correct results. It appears, therefore, that the protein digestion was not seriously disturbed during the first four days of the disease. (A similar result was obtained in the previous experiments, Rogers, 1941.) During the next

two periods, however, it was most markedly lowered. As a rule, lowest figures were recorded 4 to 8 days after infection, though previous experiments gave 8 to 12 days as the worst period. Actually, in all experiments so far carried out, 5 rats showed poorest digestion 4 to 8 days after infection and 5, 8 to 12 days after. Clinically, the lowered protein digestion was accompanied by anorexia and diarrhoea. In order to obtain a rough quantitative relationship between protein digestion and

TABLE 5.

Showing the amounts of protein digested by rats 9 and 10 before and after infection. The rats were infected at the beginning of the 5th period as indicated by the dark line.

Period	Rat 9			Rat 10		
	Intake grams.	Voided grams.	Percentage digested.	Intake grams.	Voided grams.	Percentage digested.
1	11·601	2·239	80·70	11·601	2·271	80·43
2	11·601	2·280	80·35	11·601	2·274	80·40
3	11·601	2·205	80·99	11·601	2·395	79·36
4	11·601	2·183	81·19	11·601	2·399	79·32
5	11·601	2·434	79·02	11·601	2·398	79·32
6	11·112	2·009	72·92	11·601	3·174	72·64
7	11·601	2·692	76·80	11·601	2·852	75·42
8	11·601	1·934	83·33	11·601	2·263	80·50
9	11·601	2·050	82·33	11·601	2·310	80·04
10	11,601	1·958	83·13	11·601	2·230	80·78
11	11·601	1·940	83·28	11·601	2·144	81·52
12	11·601	1·972	83·00	11·601	2·250	80·61
13	11·601	2·140	81·56	11·601	2·391	79·40
14	11·601	1·991	82·85	11·601	2·394	79·36
15	11·601	2·160	81·38	11·601	2·140	81·57
16	11·601	2·051	82·33	11·601	2·174	81·25
17	11·601	1·980	82·94	11·601	2·044	82·38
18	11·601	1·927	83·38	11·601	2·064	82·21
19	11·601	2·055	82·30	11·601	2·020	82·61

size of infection, Table 6 has been prepared. In determining the degree of digestive disorder the average of the figures for the pre-infection periods of each rat was taken as the normal level. The fall from this level for the first 3 periods of infection was then calculated and the sum of these amounts (3 for each rat) was considered to give a measure of the digestive disturbance for each animal. In Table 6 these amounts are given as the " Percentage fall in digestion."

TABLE 6.

Showing the relationship between digestive disorder and the number of infective larvae fed. Figures for rats 1, 2, 3 and 4 were taken from previous results (Rogers, 1941). For further explanation see text.

No. of rat.	No. of larvae fed.	Percentage fall in digestion.	Percentage fall in digestion per 100 larvae.
1	640	6·00	0·9
2	680	9·53	1·4
3	680	5·79	0·9
4	680	7·29	1·1
5	830	14·22	1·7
6	830	12·61	1·5
7	2,100	21·84	1·0
8	2,100	25·80	1·2
9	1,200	12·91	1·1
10	1,100	12·04	1·1

It is surprising that the percentage fall in digestion per 100 infective larvae fed should be so constant when the number of factors (immunity and diet of the host, viability of larvae, etc.) affecting these results are considered. As the amounts of infective tissue fed varied widely in quantity (rat 1 received 0·37 g., rats 2, 3 and 4, 0·40 g., the remainder, see Table 1) and not necessarily in proportion to the number of contained larvae, Table 6 supports the contention that the digestive disorders were caused by *T. spiralis*, and further, that although muscular infection was not established in rats 9 and 10, intestinal infection was achieved.

It is clear that after the first three periods of infection, the adult

parasites had no obvious effect on protein digestion though a slight fall was frequently noted about the 8th period of infection.

Daily Nitrogen Balance.

Urinary N was determined by the Kjeldahl method as before. Tables 7, 8 and 9 show the calculated daily N balances. The figures giving the balance for period 5, are, except in Table 8, somewhat low because no allowance was made for the addition of flesh to the ration. The "Intake" shown in the tables gives the nett N intake per day calculated by substracting the N per day in the faeces from the N per day taken in with the food.

Table 7.

Showing the daily N balance for rats 5 and 6. Infective larvae were given at the beginning of the 5th period as indicated by the dark line. For further explanation see text.

Period	Rat 5.			Rat 6		
	Intake mgs.	Urinary output mgs.	Balance mgs.	Intake mgs.	Urinary output mgs.	Balance mgs.
1	170	143	+27	173	126	+47
2	172	144	+28	171	130	+41
3	180	139	+41	180	148	+32
4	167	134	+33	178	145	+33
5	163	125	+38	151	108	+43
6	156	108	+48	165	122	+43
7	171	134	+37	178	119	+59
8	173	144	+29	177	142	+35
9	179	124	+55	177	150	+27
10	180	116	+64	183	151	+32
11	184	125	+59	188	168	+20
12	175	115	+60	179	150	+29
13	171	84	+87	179	99	+80
14	172	91	+81	180	112	+68
15	176	88	+88	177	78	+99

TABLE 8.

Showing the daily N balance for rats 7 and 8. The animals were infected at the beginning of the 4th period as indicated by the dark line. For further explanation see text.

Period	Rat 7			Rat 8		
	Intake mgs.	Urinary output mgs.	Balance mgs.	Intake mgs.	Urinary mgs.	Balance mgs.
1	368	305	+63	367	345	+22
2	367	327	+40	364	354	+10
3	368	330	+38	365	340	+25
4	360	320	+40	383	329	+54
5	245	250	−5	290	282	+8
6	271	222	+49	270	223	+47
7	363	317	+46	354	323	+31
8	369	366	+3	366	327	+39
9	366	368	−2	366	324	+42
10	360	329	+31	360	327	+33
11	368	355	+13	365	311	+54
12	362	345	+17	366	308	+58
13	367	332	+35	367	315	+52
14	362	334	+28	366	327	+39
15	363	345	+18	363	341	+22

Immediately after infection the urinary N output fell. This was partly due to a reduction in protein assimilation giving rise to a reduction in urea excretion. In the case of rats 5, 6, 9 and 10 where the water intake was limited the N output was further reduced because the volumes of urine passed probably fell below the augmentation limit (see Table 10) causing an even lower urea excretion. This retention then gave rise to the increased N balance. Water intake was not limited, however, in the case of rats 7 and 8, and the urine volumes did not fall greatly (see Table 10) and hence the N retention did not occur and a lowered N balance resulted.

TABLE 9.

Showing the daily N balance for rats 9 and 10. The animals were infected at the beginning of the 5th period, as indicated by the dark line. For further explanation see text.

Period	Rat 9			Rat 10		
	Intake mgs.	Urinary output mgs.	Balance mgs.	Intake mgs.	Urinary output mgs.	Balance mgs.
1	375	300	+75	373	316	+57
2	373	305	+68	373	301	+72
3	376	314	+62	369	301	+68
4	376	299	+77	368	308	+60
5	367	314	+53	368	324	+44
6	344	240	+104	337	273	+64
7	357	299	+58	350	271	+79
8	386	349	+37	373	342	+31
9	384	328	+56	372	353	+19
10	386	325	+61	376	345	+31
11	387	340	+47	378	367	+11
12	385	346	+39	374	349	+25
13	379	342	+37	368	369	−1
14	384	315	+69	368	339	+29
15	378	321	+57	379	365	+14
16	382	345	+37	377	345	+32
17	385	355	+30	382	377	+5
18	387	364	+23	381	349	+32
19	381	357	+24	383	345	+38

URINE VOLUMES.

Table 10 lists the amounts of urine passed by rats 7, 8, 9 and 10. Rats 9 and 10 were each limited to an intake of 25 mls. of liquid water per day whereas rats 7 and 8 were given water ad lib. After a fall in the

output on the 3rd day of infection (not shown in Table 10 for immediately after infection the output rose) the volume rose slightly then fell again to give a minimum about the 8 or 9th day. From this point the volumes steadily increased. In the case of rats 5 and 6, however, it was found that the urinary volumes decreased after 8 periods. It is considered that these results differed from those of rats 9 and 10 (which were also limited in water intake) because muscle infection was, in comparison, very heavy and water was retained as a result of the tissue destruction.

TABLE 10.

Showing the volumes of urines passed. Rats 7 and 8 were infected at the beginning of the 4th period, rats 9 and 10 at the beginning of the 5th.

Period	Rat 7 ml.	Rat 8 ml.	Rat 9 ml.	Rat 10 ml.
1	69	82	51	39
2	75·5	79	51	39
3	75	70	46	40
4	68	73	46	38
5	62	65	50	39
6	58	67	57	42
7	86	88	41	27
8	93	78	37	40
9	88	81	61	52
10	95	92	59	47
11	96	100	55	48
12	99	104	58	55
13	100	103	64	55
14	100	100	59	55
15	98	93	55	49
16	—	—	62	61
17	—	—	65	59
18	—	—	68	66
19	—	—	69	66

Phosphate Excretion.

Shearer and Stewart (1932) found that nematodes interfered with phosphate metabolism of sheep, retention and nett absorption increasing when infections fell. It was decided, therefore, that though material was not available for the determination of a complete P balance, the rate of P excretion in urine might give some indication as to the metabolism of infected rats, particularly in relation to urine *p*H. (The urine of rats 7 and 8, the only ones tested, was after 4 days of infection, of a higher *p*H than 7·2, determinations being made colorimetrically and with quinhydrone electrodes.) Inorganic P in urine was estimated by precipitation as strychnine molybdophosphate (Embden, 1921). The results are summarised in Table 11.

Table 11.

Showing the amounts (mgs.) of P found each period in the urines of rats 7 and 8. The rats were infected at the beginning of the 4th period.

Period	Rat 7	Rat 8
1	52·9	73·9
2	86·0	71·1
3	64·1	85·4
4	68·1	42·0
5	13·2	22·3
6	41·0	45·5
7	84·7	72·0
8	87·8	62·1
9	79·1	63·4
10	76·0	58·9
11	75·6	68·1
12	121·9	79·2
13	133·1	146·8
14	126·0	119·9
15	149·8	111·8

Rat Weights.

The experimental animals were weighed on the 2nd day of each period before being fed. Results are shown in Table 12. It can be seen that there was a minor fall in weight about the 4th or 5th period, then, following a short period of recovery, the weights fell rapidly and continuously, usually from the 7th period after infection to the 14th.

Table 12.

Showing the changes in the weights of rats before and after infection. Rats 5, 6, 9 and 10 were infected at the beginning of the 5th period, rats 7 and 8 at the beginning of the 4th. All weights are given in grams.

Period	Rat 5	Rat 6	Rat 9	Rat 10	Rat 7	Rat 8
1	122	133	166	155	167	171
2	124	136	168	159	168	173
3	128	139	174	159	168	173
4	141	141	180	160	169	174
5	131	148	186	163	177	181
6	132	145	190	166	166	166
7	130	144	188	165	166	167
8	130	147	191	167	166	170
9	129	144	189	164	164	174
10	126	141	188	166	164	173
11	125	140	190	170	162	173
12	125	140	190	167	159	169
13	130	144	188	167	156	167
14	126	144	185	166	156	166
15	123	139	187	166	152	161
16	—	—	186	165	—	—
17	—	—	185	165	—	—
18	—	—	183	164	—	—
19	—	—	185	165	—	—
20	—	—	186	165	—	—

During the period 4 to 8 days after infection there was usually a rise in weight. This was particularly notable in rats 7, 8, 9 and 10 and was usually accompanied by a marked swelling of tissues round the eyes and at the base of the nose. Similar oedematous conditions have been noted in rats (Lehmensick and Senadesaya, 1941) and man (Carter, 1930, Beeson, 1941) infected with *T. spiralis*. In the latter case orbital oedema occurs within 7 days of infection and lasts about 3 days. In the present experiments the oedema was frequently noted about 5 days after infection and sometimes lasted for 5 days.

Assimilation of Calcium.

In view of the findings of Shearer and Stewart (1932) that Trichostrongyle parasites of sheep interfere with Ca metabolism, the rate of assimilation of Ca and the daily Ca balance before and after infection with *T. spiralis* have been determined for rats 7 and 8. Ca in the faeces was determined by the titration method of McCrudden (1910, 1911). In order to estimate the urinary Ca, the titration method of Shohl and Pedley (1921) was used. Ca in the food was determined by the same method, 2 samples being examined about every 12 days. Though the variation in the Ca content of the food was fairly wide, the changes were not consistent and the results were averaged in estimating the period ration. The average figure for the Ca content of the food was $0{\cdot}792\% \pm 0{\cdot}066\%$. When some food was not eaten as in periods 5 and 6, figures for Ca intake were computed in the same way as protein intake (see section on diet).

Table 13 summarises the results obtained. " Voided " indicates the amounts of Ca in the faeces and " Intake " refers to that taken in with the food. No allowances has been made for the addition of flesh to the diet in period 4.

It would appear that the parasites interfered more with the assimilation of Ca than with the digestion of protein. Thus, after the initial disorder, the Ca intake, instead of returning to normal and remaining so, as was the case with protein digestion, fell off steeply after the 13th period. It seems possible that though the initial fall in intake was due to adult parasites in the intestine, later changes may have been caused by the larvae.

TABLE 13.

Showing the amounts of Ca assimilated by rats 7 and 8 before and after infection. The animals were infected at the beginning of the 4th period. For further explanation see text.

Period	Rat 7			Rat 8		
	Intake mgs.	Voided mgs.	Percentage assimilated	Intake mgs.	Voided mgs.	Percentage assimilated
1	351·5	262·8	25·1	351·1	277·3	21·1
2	351·5	270·1	23·1	351·1	267·4	24·0
3	351·5	254·0	27·7	351·1	268·1	24·6
4	351·5	268·1	23·8	351·1	281·8	19·8
5	287·8	283·0	6·5	324·5	309·4	5·0
6	307·6	230·2	25·2	298·7	244·2	18·1
7	361·5	277·2	21·1	351·1	271·0	22·9
8	351·5	271·1	27·8	351·1	312·1	11·1
9	351·5	280·7	20·1	351·1	296·5	15·6
10	351·5	282·4	19·5	361·1	288·4	17·8
11	351·5	289·2	17·6	351·1	291·7	17·0
12	351·5	286·0	18·5	351·1	269·0	23·5
13	351·5	261·5	26·6	351·1	265·6	24·5
14	351·5	290·2	17·4	351·1	319·0	9·2
15	351·5	312·9	11·0	351·1	324·2	7·6

DAILY CA BALANCE.

The Ca balances for rats 7 and 8 are shown in Table 14. " Intake " refers to the amounts taken in with the food minus that excreted in the faeces. " Output " gives the amounts excreted in the urine. Again no allowance has been made for the infective flesh fed in the ration of period 4 and the balance figures given are lower than the true results for this period.

TABLE 14.

Showing the daily Ca balances for rats 7 and 8. The animals were infected at the beginning of the 4th period. For further explanation see text.

Period	Rat 7			Rat 8		
	Intake mgs.	Output mgs.	Balance mgs.	Intake mgs.	Output mgs.	Balance mgs.
1	22·2	8·0	+14·2	18·5	7·3	+11·2
2	20·2	7·7	+12·5	21·0	8·0	+13·0
3	24·4	7·6	+16·8	20·9	7·5	+13·4
4	20·9	6·5	+14·4	17·4	6·4	+11·0
5	1·2	3·9	— 2·7	3·5	3·3	+ 0·2
6	19·3	7·3	+12·0	13·7	5·0	+ 8·7
7	18·6	8·8	+ 9·8	20·1	6·1	+14·0
8	20·1	6·4	+13·7	9·9	5·5	+ 4·4
9	17·7	8·8	+ 8·9	13·8	3·3	+10·5
10	17·3	8·3	+ 9·0	15·8	4·5	+11·3
11	15·6	9·2	+ 6·4	15·0	2·7	+12·3
12	16·4	8·9	+ 7·5	20·6	6·8	+13·8
13	22·5	9·0	+13·5	21·5	7·3	+14·2
14	15·3	9·2	+ 6·1	8·1	6·8	+ 1·3
15	9·7	8·9	+ 0·8	6·8	6·5	+ 0·3

HISTOLOGICAL STUDIES.

The nature of the pathological changes occurring in the intestine, heart, kidney and liver of rats infected with *T. spiralis* was examined. A number of rats were each infected with about 1,000 larvae and at 4-day intervals animals were killed and portions of the organs concerned were fixed in Helley's fluid. The sections, stained with haematoxylin-eosin, were examined by Professor Shaw-Dunn. Though kidneys taken from rats which had been infected 20 to 42 days showed some partial atrophy of tubules with round cell infiltration and some swelling of the epithelium of deeper nephrons, no marked changes which could be definitely attributed to the parasites could be found. In particular, no myocarditis of recent origin was noted. In considering these results

it must be emphasised that the infections given were, at most, moderate (see discussion).

Discussion.

The size of the infective doses used in these experiments was at first based on the work of Beahm and Downs (1939) who found that rats given more than 900 Trichinella larvae died within about 30 hours. However, in the present experiments it was found that even when infective doses of more than 1,000 larvae were used the animals did not appear to be unduly distressed and the symptoms of disorders arising from the invasion of the musculature by larvae were not marked, though digestive disturbances were sometimes severe. It would appear then, in the light of the results of Beahm and Downs (1939) that either the rats used in the present experiment were resistant, or that the virulence of the infective material was comparatively low. As different strains of rats were frequently used it would appear that the latter contention is the correct one. It is felt, therefore, that the results given in this paper relate, at most, to moderate infections only.

The results showing the effects of *T. spiralis* on protein digestion in rats obtained by Rogers (1941) have been confirmed. It would appear from the study of rats 9 and 10 (see Table 5) that the release of larvae in the host intestinal tissues was not the cause of the failure in protein digestion. Rather, it seems that this disorder was entirely due to the adult parasites, probably arising from the inflammation caused by the movements of the worms in the submucosa. This contention is well borne out by the results shown in Table 6, for the fall in digestion per 100 larvae fed was fairly constant and did not appear to depend at all on the number of larvae eventually detected in the tissues.

Besides the digestive disorder occurring immediately after infection, there was frequently a minor fall in protein digestion about 7 or 8 periods later. It is suggested that this may have been due to the large numbers of female parasites leaving the host intestinal wall at that time causing some inflammation and themselves contributing to the protein in the faeces. Faust (1939) lends support to this contention in that he states that the adult females leave the host before the 33rd day of infection. However, Cameron (1934) says that the maximum life of the females is 12 weeks.

The fall in the percentage of Ca assimilated during period 5 (see Table 13) may have been due to the indirect action of the parasites for

at that time diarrhoea was marked, and this alone would cause a decrease in absorption. However, the fall in intake during the 14th and 15th periods cannot be explained as the result of localised intestinal disorder, for the adult Trichinella probably left the host before this time. It is possible that the presence of the larvae in the host tissue caused an increased excretion of Ca or alkali in the intestine thus giving more Ca in the faeces. Urinary Ca (see Table 14) did not vary greatly, the fall in period 5 probably being caused by the lowered intake.

Though the amounts of inorganic P in the urine (see Table 11) roughly followed the urinary volumes (see Table 10) the varying excretion was not a secondary effect depending on factors influencing the urine volumes, for normal phosphate excretion is not changed by water diuresis (Harvard and Reay, 1926) and is only slightly affected by salt diuresis (Hart and Verney, 1934). The fall in P excretion during the 5th period was probably due to a reduced intake in the intestine at a time when the digestive disurbance was at a maximum. Following the 11th period, the increased excretion was probably the result of tissue breakdown caused by the larvae in the muscles, for endogenous P excreted in urine is usually obtained from body tissues and is a maximum in conditions of increased metabolism and fevers.

Faust (1938) has been able to divide the course of trichinosis in man into three phases. First, the " incubation " period, lasting about seven days, in which the primary symptoms are those of gastro-intestinal disorder. The second or " acute " stage is the period of migration of the larvae and is marked by a more or less profound myositis. Oedema and dypsnea may be intense and the temperature rises. The final " chronic " stage is the critical one and marks a period of larval encystment and host tissue repair. Oedema may persist and a cachexia " due to the absorption of toxins from the larvae " develops.

It is difficult to detect these three phases in infected rats. The " incubation " period seems to be longer and the various stages seem to merge. Thus the stage of gastro-intestinal disorder occurs four to twelve days after infection and is well defined though oedema occurs only during this time. The middle of this period is indicated by the lowering of urea, creatinine and creatine excretion and protein and Ca assimilation reaches a minimum. The next phase of the disease seems to be a merging of Faust's " acute " and " chronic " stages. Increased urea, creatinine, creatine, inorganic P and Ca excretion and a

steady fall in weight at the end of this period indicates that it is probably the result of tissue invasion followed by the encystment of the larvae causing even more host tissue destruction.

Summary.

1. The action of *Trichinella spiralis* infections in markedly lowering protein digestion in rats is confined to a period 4 to 12 days after infection. Minor disturbances in protein digestion occurring about 30 days after infection may have been due to adult females leaving the host.

2. Over the first 12 days of infection the protein digestion was lowered (calculated as the summation of the percentage fall for each of the first 3 periods) approximately 1% for each 100 infective larvae fed. The degree of digestive disorder seemed unrelated to the number of larvae finally recovered from the hosts' tissues.

3. Inorganic P excretion fell to a very low level 4 to 8 days after infection. Twenty-four days later the rate of excretion rose, the maximum being 2 or 3 times the normal level.

4. Ca assimilation fell to a very low point during the second period of infection, falling again after a further 9 periods.

5. As indicated by weight losses the most marked period of the disease occurred from the 8th to the 11th period after infection.

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